EVALUATION STUDIES ON A CANINE ACUTE PHASE SERUM FRACTION ANALOGOUS TO HUMAN C-REACTIVE PROTEIN

by

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INTRODUCTION

Medical diagnostic and prognostic laboratory tests and determinations have played an ever-increasing role in the advance of the medical sciences. Common clinical laboratory procedures probably have saved countless lives and eliminated needless suffering in the practice of human medicine. The veterinary medical profession has been slow to adopt these procedures in some instances. Laboratory tests and procedures which are used in human medicine frequently cannot be adopted for use in veterinary medicine because of innate species differences. When procedures are found that are applicable to animals, the cost often is prohibitive. Whenever diagnostic and prognostic tests and procedures can be adopted to the practice of veterinary medicine, they have been found to be of value.

In human medicine, various tests have been devised to detect the presence of a non-specific protein in the serums of patients during the acute phase of a pathological process. This protein fraction, designated "C-reactive protein" by MacLeod and Avery (1941a), has been detected by the original precipitation procedure of Tillet and Francis (1930), a non-specific capsular swelling reaction devised by Löfström (1942, 1943), and MacLeod and Avery's method (1941a) of precipitation with a rabbit antiserum specific for C-reactive protein. In addition to these original methods for detection of C-reactive protein, various modifications have developed in an attempt to increase the sensitivity of the test.

Anderson and McCarty (1951) demonstrated a protein fraction in the "acute phase" serums of rabbits which was analogous to the

human C-reactive protein. Gotschlich and Stetson (1960) further demonstrated cross-reacting "acute phase" proteins in the serums of man, monkey, and the rabbit. Gotschlich (1962) described a substance present in "acute phase" dog serums analogous to human C-reactive protein and which would cross-react with the human serum fraction.

The purpose of this work was to study the canine serum fraction and learn something of its physical properties and occurrence during inflammation. This was accomplished by agglutination reactions involving latex-adsorbed rabbit antiserum, specific for human C-reactive protein, to detect the cross-reacting canine serum fraction. The extent of the inflammatory processes was determined by hematologic analyses and observation of gross and microscopic lesions at the end of each experimental period. The stability of canine C-reactive protein in refrigerated, frozen, and heat treated serum samples was assessed and its association with known serum fractions evaluated.

REVIEW OF LITERATURE

In 1930, Tillet and Francis (1930) detected "precipitins" in the serums of patients with an acute phase pneumonia which produced a precipitation reaction when pneumococcal C-polysaccharide fraction was added to the serum. The reaction did not occur in serums from patients who had passed the crisis stage of the disease nor in clinically normal individuals. These workers further demonstrated this reactive substance in the serums of patients acutely ill with rheumatic fever, bacterial endocarditis,

and staphylococcal osteomyelitis. It was noted that "anti-pneumococcus sera from animals in some instances precipitated the C Fraction." Since all of the cases involved Gram positive microorganisms, it was theorized that the "precipitins" were antibody-like substances specific for Gram positive microorganisms. It was of particular interest to them that the "precipitins" appeared early in the course of the disease and disappeared after the acute phase of the infection.

A few years later, Ash (1933) extended the observations of Tillet and Francis to include diseases produced by Gram-negative bacteria and used the term "C-reactive substance" to describe the serum fraction responsible for the precipitation reaction with the pneumococcal polysaccharide C fraction. She noted a relationship between the precipitation reaction, fever, and sedimentation rate, but found there was a marked individual variation in the reactions.

Later, Löfström (1939) described a substance which, under certain conditions, occurred in human serums and caused a non-specific swelling of the capsules of pneumococci of types 27 and 28, and occasionally of other types. He called the substance "non-specific capsular swelling substance" and demonstrated its presence in the serums of patients with acute pneumonia and in cases of respiratory tract infections with secondary bacterial complications. The reaction was called a "non-specific capsular swelling reaction (CRS)." He observed a similarity between the occurrence of CSR and the precipitation reaction produced by the C-reactive substance described by Tillet and Francis (1930), and

later by Ash (1933).

The first investigations of the biochemical properties of the C-reactive substance were performed by Abernathy and Avery (1941) who described its protein nature. They found that the substance in diluted serum was inactivated after exposure to heat in excess of 65 C. They determined that the C-reactive substance was carried down with the serum albumin fraction when salting out procedures with ammonium or sodium sulphate were used. They also made the observation that calcium ions were needed for the C-reactive substance to precipitate the pneumococcal C fraction. This evidence emphasized the difference between this reaction and an antigen-antibody reaction. MacLeod and Avery (1941) noted that calcium and lipids decreased the solubility of the "reactive protein." MacLeod and Avery (1941a) found the reactive protein to be highly antigenic in rabbits. They first used the phrase "C-reactive protein" (CRP) in describing its properties; the term which is still in use today. Rabbit antibodies against the CRP were found to react specifically with purified CRP as well as with acute phase serums from humans and monkeys having bacterial infections. The immunological specificity of the rabbit CRP-antiserum (CRPA) was demonstrated by precipitation and complement fixation tests. The sensitivity of the precipitation test for CRP was higher with antiserum than with the pneumococcal C-polysaccharide used in the original precipitation reaction reported by Tillet and Francis (1930).

Various serum analyses have been made in an attempt to establish the identity of CRP. Perlman et al. (1943) found that

CRP migrated with the alpha globulin fraction. Löfström (1943) established that the non-specific capsular swelling substance, which produced his "capsular swelling reaction (CSR)," belonged to the globulin fraction and moved faster than the gamma globulins. He later reported that this "non-specific capsular swelling substance" was identical with C-reactive protein (Löfström, 1944). Wood et al. (1954) found that crystalline CRP migrated as a beta globulin. Hedlund and Brattsten (1955, 1956) believed that the acute phase protein in human serum migrated with the gamma globulins. Hedlund (1958) suggested that CRP consists of at least two fractions. Fishel et al. (1960) confirmed this finding and suggested the possibility of a third CRP fraction. Libretti et al. (1955) found three distinct bands when purified CRP was allowed to diffuse in agar against rabbit CRPA. Early in these investigations, a possible reason for this confusion was suggested by Hedlund (1947) who felt that the extremely small quantities of CRP necessary for the precipitation reaction made it difficult to associate with known serum fractions.

Purified crystalline CRP was first produced by McCarty (1947) from human serous fluids. He also demonstrated the antigenic properties of purified crystalline CRP in rabbits and the specificity of the antiserum so produced, thereby confirming the earlier findings of MacLeod and Avery (1941a). Production of a purified source of highly antigenic CRP was an important step in the development of a test for clinical evaluation of CRP in man. Wood et al. (1954) modified the technique and obtained a high yield of the crystalline CRP from ascitic fluid in advanced

cases of neoplasia.

Several tests for clinical detection and evaluation of CRP evolved from this work. The original precipitation reaction of Tillet and Francis (1930), using pneumococcal C-polysaccharide, was used in the early evaluation studies. The non-specific capsular swelling reaction (CSR) of pneumococci types 27 or 28, developed by Löfström for clinical evaluation studies (Löfström, 1942, 1943), also was used. With the advent of MacLeod and Avery's sensitive precipitation reaction, employing rabbit antiserum specific for CRP (MacLeod and Avery, 1941a), clinical evaluations became more popular. Anderson and McCarty (1950) adapted a capillary tube precipitation procedure, similar to that used in the serological typing of group A hemolytic streptococci, for use with rabbit CRPA in the detection of CRP. They performed the first extensive clinical study concerned with a single disease. They concluded that the CRP test was "the most sensitive factor" in the laboratory evaluation of acute rheumatic fever and associated disease processes.

With this development of the capillary tube test for routine clinical studies, many other extensive clinical evaluations of CRP in various diseases were performed. Shetlar et al. (1955) studied serum CRP, glycoprotein, and seromucoid in cancer, arthritis, tuberculosis, and pregnancy. Roantree and Rantz (1955) studied CRP in 443 general and 50 control cases and concluded that "The C-reactive protein test gave fewer false positive reactions than the other indices of inflammation." They also felt that "A distinct advantage of the C-reactive protein test is that no

interpretation of normal range is necessary. Any positive reaction is considered abnormal." Wood (1958) found the CRP test of value in following the course of some forms of Hodgkin's disease. The Russian workers Shmerling and Pashinin (1961) recommended the use of the CRP test in following the course of infectious hepatitis. The Russian worker Kabakov (1961) noted a correlation between CRP levels and the various forms of leprosy studied, and suggested that further investigations be performed. Heiskell et al. (1962) noted a correlation between the smoking habits of 963 females and 549 males and the appearance of CRP in clinically "normal" smokers. Parker et al. (1962) evaluated the CRP test in viral infections and concluded that it was of limited value.

Since the development of the capillary precipitation test for CRP by Anderson and McCarty (1950), workers have attempted to increase the sensitivity of the test and make it simpler to perform. Singer et al. (1957) developed a latex particle agglutination test which produced results that correlated well with those obtained by the capillary precipitation procedure. Although the sensitivity is not increased, the test is much simpler to perform and evaluate. A commercial modification of this technique is available.* Fukuda et al. (1959) developed a quantitative gel diffusion technique for the determination of CRP. Goldwasser and Rozonsky (1961) described a method of CRP detection employing fluorescent antibody techniques.

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* CR-TEST, Hyland Laboratories, Los Angeles 39, California.

A serum fraction, analogous to human CRP, was observed in monkeys as early as 1937 (Abernathy, 1937). Using a highly purified pneumococcal polysaccharide, which they called "Cx-polysaccharide," Anderson and McCarty (1951) detected an acute phase protein in the serum of the rabbit. They called this rabbit serum fraction "Cx-reactive protein" (CxRP) after the human serum counterpart. Like the human CRP, CxRP was not present in the serums of normal rabbits, but did appear early in the course of an acute inflammatory condition and disappeared upon recovery. Calcium was found to be necessary for the reaction. Wood (1953) found a noticeable individual variation in the ability of rabbits to produce CxRP. He noted that rabbits that consistently produced high levels of antibody also produced high CxRP levels. In studying the site of production of human CRP, Montella and Wood (1957) were able to prevent CxRP production in the rabbit by blocking the reticuloendothelial system with thorodrast injections.

Gotschlich et al. (1960) observed cross reactions among CRP-like fractions in man, monkey, and the rabbit. They concluded that the acute phase proteins of man, monkey, and the rabbit were very similar, but not identical. Wood et al. (1960) were able to produce CxRP in the rabbit by exposure to X-rays. Hokama et al. (1960) produced CxRP in the rabbit using foreign protein inoculations. They were able to inhibit CxRP formation in the rabbit by the injection of cortisone. Hedlund (1961) reported low titers of CRP-like fractions in the serums of mice, hens, and guinea pigs. Gotschlich (1962) described a substance in the acute phase

serums of the canine analogous to human CRP, and discussed the cross-reactions occurring between them.

MATERIALS AND METHODS

Test Animals and Experimental Groups

Five litters, comprising a total of 31 dogs, were used in the experiments. The animals were from 16 weeks to 1 year of age. Twelve of the animals were females and 19 were males. Eleven of the dogs were Beagles and 20 were mongrels.

Littermates were assigned sequential numbers and identical letters to facilitate the identification of litters and individuals. The designations are presented in Table 1.

TABLE 1. Experimental Dogs' Identification

Dog breed or type	Litter size	Individual identification
Beagle	6	1B, 2B, 3B, 4B, 5B, 6B
Beagle	5	1X, 2X, 3X, 4X, 5X
Mongrel	10	1M, 2M, 3M, 4M, 5M, 6M, 7M, 8M, 9M, 10M
Mongrel	6	2MM, 3MM, 4MM, 5MM, 6MM, 9MM
Mongrel	4	1S, 3S, 5S, 7S
Total dogs	31	

Fecal examinations were performed on all dogs prior to the experimental period. Ascarid and hookworm ova were demonstrated. The dogs were treated once intravenously with disophenol* and twice orally with di-phenthane-70 in combination with

* D.N.P., American Cyanamid Co., Princeton, New Jersey.

methylbenzene* prior to the experimental period. No ova were then observed on fecal flotation.

Wood et al. (1960) observed that the injection of antibiotics into normal rabbits, via various routes, produced enough inflammation to cause CxRP to appear in the serums. Hedlund (1961) observed CxRP in an occasional rabbit as the result of trauma induced during cardiac puncture. In consideration of these facts, a "vena puncture control" dog was included in Groups I, II, III, and VI. The dogs in Groups IV and V also were being tested on another project and vena puncture controls could not be included. The vena puncture control dogs received no inoculations but were bled with their mates in the group.

The dogs were divided into 6 experimental groups. Whenever possible, littermates were used in an experimental group. Groups IV and V were composed of the same dogs. The 6 groups were subjected to the following experimental conditions:

Group I—This group consisted of dogs 1B, 2B, 4B, and 6B. Dog 1B was inoculated subcutaneously with 10 ml. of sterile skimmed milk. Dog 4B was inoculated subcutaneously with 10 ml. of hog cholera antiserum. A sterile needle was passed subcutaneously into dog 2B and no inoculum was given. Dog 6B was the vena puncture control and was not inoculated.

Group II—This group was composed of dogs 1S, 3S, 5S, and 7S. Thiamylal sodium** was administered intravenously to dogs 1S and

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* Vermiplex, Pitman-Moore Co., Division of Allied Laboratories, Inc., Indianapolis, Indiana.

** Surital Sodium, Parke-Davis, Detroit, Michigan.

3S until a level of anesthesia was obtained where a cough reflex could not be elicited. The dogs were placed on their sternums and a polyethylene tube with a 5-mm. outside diameter was passed into the primary bronchi first on one side and then the other. A 10-ml. syringe, filled with sterile mineral oil, was attached and 5 ml. of the oil deposited in each bronchus. Dog 5S also was given anesthetic, and another polyethylene tube passed into each bronchus, but no sterile mineral oil was administered. Dog 7S served as a vena puncture control.

Group III—Dogs 1X, 2X, 3X, 4X, 3B, and 5B were in this group. Dogs 1X, 2X, 3B, and 5B each were inoculated intraperitoneally with a 10-ml. sterile suspension of 2.5 grams of talcum powder in physiologic saline. Dog 3X was inoculated intraperitoneally with 10 ml. of sterile physiologic saline solution. Dog 4X served as the vena puncture control.

Groups IV and V—The same 10 dogs comprised both of these groups and were test animals from a measles-distemper research project. Group IV consisted of dogs 1M through 10M. Dogs 2M, 3M, 4M, 5M, 6M, 7M, and 9M were inoculated subcutaneously with 2.0 ml. of an undiluted suspension of canine-adapted human measles virus in tissue culture fluid. Dogs 1M and 8M were inoculated with 2.0 ml. of tissue culture fluid, and dog 10M was not inoculated.

Group V animals were those dogs from Group IV that were challenged with virulent distemper virus one month following vaccination with the canine-adapted human measles virus. Dog 7M succumbed to a bacterial enteritis in the interim. All

animals in Group V were challenged intracranially with .5 ml. of a 1:50 dilution of the Snyder-Hill strain of distemper virus in canine brain tissue.

Group VI—This group was composed of dogs 2MM, 3MM, 4MM, 5MM, 6MM, 9MM, and 5X. Dogs 2MM and 9MM were inoculated subcutaneously with 10 ml. of a 24-hour nutrient broth culture of hemolytic Escherichia coli at a concentration of 20 x 10^6 cells/ml., as determined by a bacterial plate count. The same quantity of this culture was injected intraperitoneally into dogs 4MM and 6MM. Dog 3MM was inoculated subcutaneously with 10 ml. of sterile nutrient broth, and dog 5MM received 10 ml. of sterile nutrient broth by intraperitoneal inoculation. Dog 5X was the vena puncture control animal and was not inoculated.

Aseptic techniques were observed in making all inoculations. Hair was clipped from each injection site. The skin was washed with liquid soap and rinsed with tap water. A 70% ethyl alcohol solution was applied and allowed to dry for approximately 1 minute. The skin was saturated with the alcohol solution again and allowed to dry for 30 seconds. A sterile needle and syringe were used for each inoculation. During the postmortem procedures, the inoculation sites were examined for bacteria by swabbing them with sterile swabs and culturing the swab contents on blood agar.

Gross and Histologic Examination

With the exception of Groups IV and IV, which were involved in another project, dogs were euthanatized and necropsied 7 days after inoculation. Special emphasis was placed on the examination

of grossly inflamed tissues. Tissues from all test and control animals were sectioned at 5 microns, stained with hematoxylin and eosin, and examined. In addition, frozen sections of lung tissue from Group II animals were stained with Oil-Red-O for confirmation of the mineral oil content.

Rectal Temperatures

Rectal temperatures were taken just prior to the withdrawal of blood on the collection day. All temperatures were taken with the same thermometer.

Blood Collection and Collection Schedule

To facilitate the collection of blood, the hair was clipped from the skin area over both jugular and cephalic veins. This procedure was repeated when it seemed that hair growth would interfere with an aseptic vena puncture. Blood was easily obtained from the left jugular vein. When trauma from the previous withdrawals of blood was sufficient to prevent use of the left jugular vein, the corresponding vessel on the right was used. Blood was withdrawn from the cephalic veins only when the jugular veins were badly traumatized from previous blood withdrawals.

Aseptic technique was observed during the collection of the blood samples. Extremely dirty skin surfaces were washed with liquid soap and rinsed with clean tap water. Each skin surface was rubbed with 70% ethyl alcohol which was allowed to dry for approximately 1 minute before the skin was again saturated with alcohol. The vena puncture was made after allowing the second

alcohol application to dry for 30 seconds.

Vena punctures were made with sterile 21-gauge, 1½-inch needles attached to sterile 10-ml. glass syringes. Approximately 7 ml. of blood was withdrawn from each animal on the collection day. Approximately 1 ml. was placed in small glass tubes to which had been added a quantity of 10% solution of a sodium salt of ethylenediamine tetraacetate (E.D.T.A.). The remaining 6 ml. of blood was placed in larger glass tubes where it was allowed to clot.

All blood samples were collected between 7:00 and 10:00 a.m. Blood was collected from Groups I, II, III, and VI at 14 and 7 days prior to the inoculation day. Another sample was drawn on the day of inoculation and the other blood samples were collected 1, 3, 5, and 7 days after inoculation. The dogs in Group IV were bled 2 days and 1 day prior to inoculation. Blood was withdrawn on the day of inoculation and 2, 4, 6, 8, and 10 days after inoculation. These same dogs in Group V were bled on the day of inoculation and 2, 4, 6, 8, 10, and 12 days after inoculation.

Hematologic Examination

The unclotted blood samples were subjected to routine hematologic examination. Packed cell volumes were determined using the microhematocrit procedure. Hemoglobin values were obtained by spectrophotometric analysis* of .02 ml. of blood to which had been added 6 ml. of cyanmethemoglobin reagent. The spectrophotometer wavelength setting was 540 millimicrons. The total

* Coleman Jr. Spectrophotomer, Coleman Instruments Inc., Maywood, Illinois.

leukocyte counts were determined by the electronic counting of cells,* and this procedure was periodically checked by a hemocytometer count on random samples. Blood smears were made, air dried, and stained using the Wrights-Leishman technique as described by Schalm (1961). The stained blood films were then examined under the oil immersion lens and the different cell types counted.

Serum Collection and Processing

The clotted samples were allowed to clot at room temperature for approximately 1 hour. At the end of this time, each clot was broken away from the tube wall with a wooden applicator stick and the tubes of clotted blood placed in a centrifuge. The clotted samples were centrifuged for 20 minutes at 2000 r.p.m. Each yield of approximately 3 ml. of serum was removed by pipetting with 5 ml. serological pipettes. The serums were pipetted into glass tubes which were then placed in a water bath at 56 C. for 30 minutes to inactivate the complement (Shigekawa et al., 1963). At the end of this time, each serum sample was divided into three 1 ml. aliquots which were placed in small glass tubes for further processing. One portion was considered to be a "fresh" sample, and determinations were made immediately. Another portion was placed in a refrigerator at 4 C. overnight. The third aliquot was placed in the -20 C. freezer for periods of from 7 to 10 days.

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* Coulter Counter Model A, Coulter Electronics, 590 West 20th Street, Hialiah, Florida.

Serum Electrophoretic Analysis

The "frozen" serum samples were subjected to paper electrophoresis* after the stability of the acute phase protein in a frozen state had been determined. The stained strips were photoelectrically analyzed* and the relative per cent of each serum fraction was determined.

CRP Determinations and Stability

CRP plate agglutination reactions were used to detect the acute phase protein in the canine serums. The reagents used were those produced commercially for the detection of CRP in human serum and consisted of glycine-saline diluent and latex-adsorbed rabbit antiserum specific for human CRP.** The tests were performed on an illuminated box and ruled glass plate of the type used in testing bovine serums for Brucella antibodies.

Twofold dilutions of the serum samples were made in the glycine-saline diluent. The dilutions made were 1:2, 1:4, and 1:8. If a reaction occurred at the 1:8 dilution, additional twofold dilutions were made and tested.

The highest serum dilution was drawn by capillary action into a glass capillary pipette.** The pipette was held perpendicular to the ruled glass plate, and air pressure was applied by mouth until one drop of serum formed and fell into the ruled area. The pipette was then "blown out" on a paper towel and the

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* Spinco Model R Paper Electrophoresis System with Duostat Model RD-2 and Analytrol Model RB, Spinco Division, Beckman Instruments, Inc., Stanford Industrial Park, Palo Alto, California.

** CR-TEST, Hyland Laboratories, Los Angeles 39, California.

procedure repeated on the next lower dilution, the last sample deposited being serum from the undiluted sample. The capillary pipette was then discarded and the procedure repeated on the next series of dilutions with another capillary pipette.

One drop of Latex-Anti-C-Reactive Protein Reagent* was added to each drop of serum on the plate by means of the dropper supplied with the reagent. The latex reagent was added to the serums in the same order that they were deposited on the ruled glass plate. Wooden applicator sticks were used to mix the serum and latex reagent. The serum and reagent were mixed with a stirring motion and spread over a circular area of approximately 15 mm. diameter.

The ruled glass plate was rotated from side to side for 1 to 2 minutes and placed on the illuminated box. The highest serum dilution agglutinating with the reagent was recorded as the CRP titer of that serum sample. The strength of each agglutination reaction was rated from 1 to 4 as follows:

1+ .... weak ..... barely visible agglutination.

2+ .... moderate ..... easily visible agglutination.

3+ .... strong ..... clumping and some margination.

4+ .... very strong ..... marked clumping and margination.

A known positive human serum sample was tested with each group of canine serums as a reagent control. As the work progressed, and strongly positive canine serum samples became available, known positive canine serums were included as further controls. Known negative serum controls were not included as negative

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* CR-TEST, Hyland Laboratories, Los Angeles 39, California.

serums were present in every group tested.

Löfström (1943) demonstrated the stability of his "non-specific capsular swelling substance" by observing unchanged CSR titers in serums that had been stored for years at -20 C. Hedlund and Brattsten (1956) likewise demonstrated the stability of CRP frozen for months at -20 C. To test the stability of canine CRP under refrigerated and frozen conditions, all serum samples from Groups I, II, III, V, and VI were tested as "fresh," "refrigerated," and "frozen" samples. A "fresh" sample was a 1-ml. serum sample immediately after complement inactivation on the day of collection. A "refrigerated" sample was one that had been stored overnight at 4 C. The third 1-ml. aliquot of serum was tested after storage at -20 C. for 7 to 10 days. Serum samples from Group IV had been frozen for 4 months before being tested for CRP and were all considered "frozen" samples.

Abernathy and Avery (1941) demonstrated the heat lability and protein-like qualities of CRP. They tested a group of CRP positive serums after 30 minutes' exposure to heat at 55, 60, 65, and 70 C. and found that the CRP was inactivated by 30 minutes' exposure to a temperature of 70 C. To test the stability of canine CRP in heated serum samples, 10 CRP positive and 10 negative serum samples were selected from the stored frozen samples, after the stability in the frozen state had been determined. C-reactive protein tests were again conducted on these 20 samples and the results recorded. All 20 of the samples were then placed in a water bath at a temperature of 70 C. for 30 minutes and the CRP tests run again after the serums had

cooled to room temperature.

After completion of the CRP testing procedure, the plate was washed with triple distilled water and dried with paper towels. If the plate was to be stored for some time, it was covered with aluminum foil. Before the next series of tests, the foil was removed and the plate washed and dried again.

RESULTS

Two hundred eighty-three blood samples were collected during the experimentation periods and were subjected to the various tests and analyses previously described. Sixty-six of the serum samples were positive for CRP as determined by the agglutination reaction. A summary of the results of the analyses is presented in Appendix, Tables 1, 2, and 3. With the exception of Group IV, the CRP data presented in the Appendix, Tables 1 and 2 were obtained from "fresh" serum samples. Appendix, Table 3 is a summary of the CRP values in the "fresh," "refrigerated," and "frozen" serum samples.

Hematologic and Pathologic Results

Group I—The test dogs, 1B and 4B, received foreign protein by subcutaneous inoculation. Dog 1B received 10 ml. of sterile skimmed milk, and dog 4B was inoculated with 10 ml. of hog cholera antiserum. Control dog 2B had a subcutaneous puncture made with one of the hypodermic needles, and the vena puncture control dog was 6B.

No clinical reaction was observed in the test dogs for 24 hours. At the end of this period, a pronounced subcutaneous swelling and marked cutaneous hyperemia were observed at the site of inoculation. The swollen areas pitted upon pressure and were not painful to the animals. After 72 hours, the swelling and hyperemia disappeared. Control dogs 2B and 6B did not develop similar swellings.

A correlation of the total leukocyte count, CRP titer, and rectal temperature for Group I dogs is presented in Fig. 1.

At the peak of the inflammatory reaction, 24 hours post-inoculation, test dogs 1B and 4B were febrile, and dog 1B had a leukocytosis which persisted until 5 days after inoculation. The differential counts (Appendix, Table 1) developed only slight changes. The control dogs, 2B and 6B, did not develop a leukocytosis or fever.

Serums from both test animals, 1B and 4B, were positive for CRP at the height of the inflammatory reaction and had titers of 1:2. In addition, dog 1B had an agglutination reaction of 1+ at the endpoint (Appendix, Table 1) on the day of inoculation. Serums from control dog 2B and vena puncture control dog 6B were negative when tested for CRP.

Postmortem examination of the inoculation sites of dogs 1B and 4B, 1-week postinoculation, revealed small amounts of edema deep in the subcutaneous tissues surrounded by margins of active hyperemia. The inflamed areas were roughly circular and had diameters of approximately 3 cm. Histopathologic examination of the inflamed tissues of both test dogs revealed congested

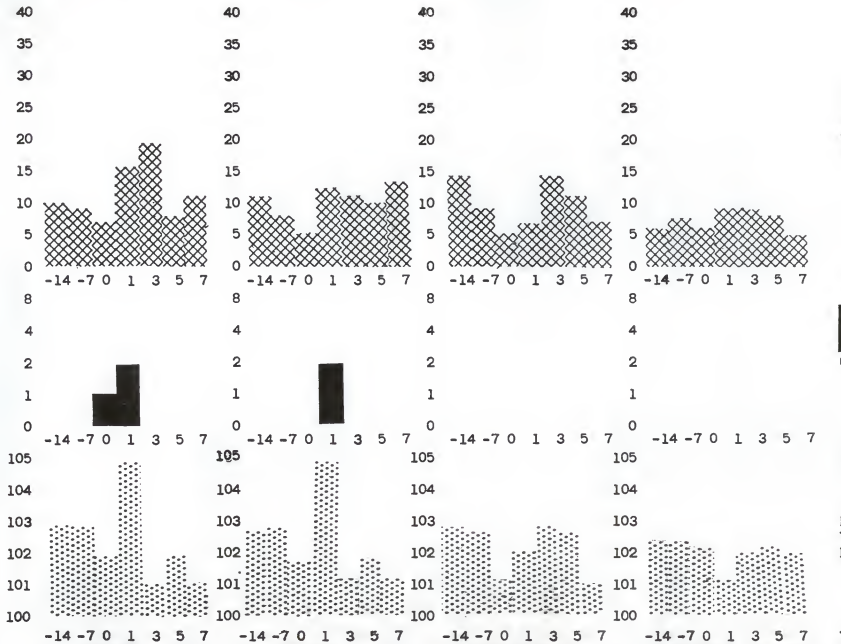


Fig. 1. A Correlation of Total White Cell Count, CRP Titer, and Rectal Temperature for Dogs in GROUP I.

subcutaneous vessels with pink staining serous fluid present interstitially. A diffuse infiltration of lymphocytes and plasma cells was present, and an occasional large mononuclear cell was observed. No gross or microscopic lesions were seen at the needle puncture site in control dog 2B. No gross or microscopic lesions were seen in vena puncture control dog 6B.

Group II—Test dogs 1S and 3S were anesthetized with thiamylal sodium.* While in a sternal position, 5 ml. of sterile mineral oil was deposited in the primary bronchi of each lung in each dog via a polyethylene tube. The same procedure was performed on control dog 5S, but no mineral oil was administered. Dog 7S served as the vena puncture control animal.

In approximately 7 minutes, the 3 dogs again were alert, moved about, and coughed spasmodically. Ten minutes later, the deep coughing had decreased in severity and by the end of 20 minutes, the animals had stopped coughing. Throughout the remainder of the test period, no coughing was observed and the animals appeared clinically normal, although moist rales were heard during the first 24 hours in those dogs which had received mineral oil. The vena puncture control animal 7S was clinically normal during the experimental observation period.

A correlation of the total leukocyte count, CRP titer, and rectal temperature for the dogs in Group II is presented in Fig. 2.

Test dog 1S developed a slight leukocytosis 5 days post-inoculation, but the other test animal 3S did not. Control dog 5S, which had a polyethylene tube passed into the bronchi but

* Surital Sodium, Parke-Davis, Detroit, Michigan.

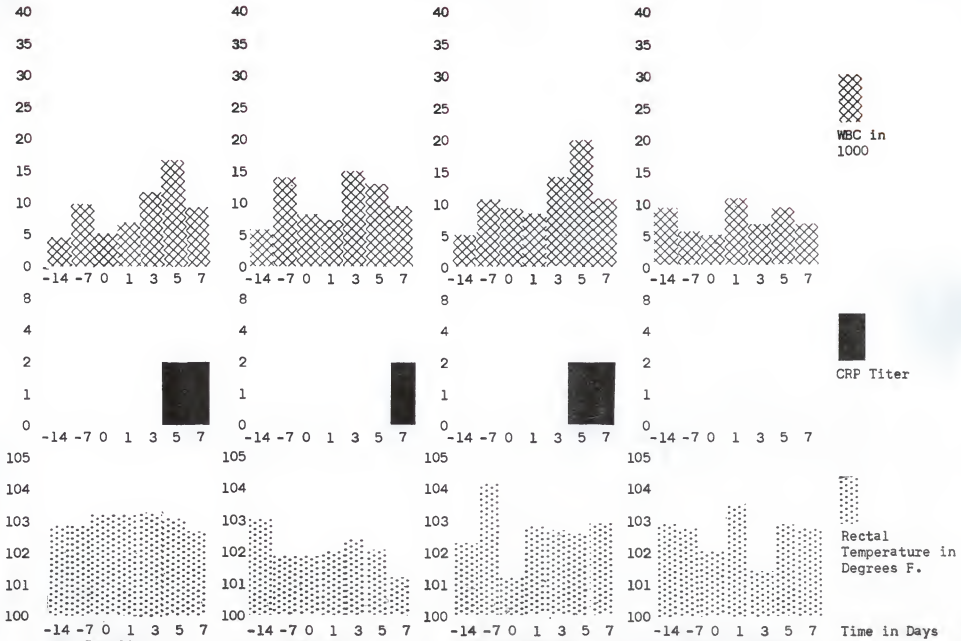


Fig. 2. A Correlation of Total White Cell Count, CRP Titer , and Rectal Temperature for Dogs in GROUP II.

received no mineral oil, did develop a leukocytosis in 5 days. None of the dogs in this group were febrile the day of inoculation nor later in the test period. Control dog 5S had a temperature of 104.2 seven days prior to the inoculation day, but had a temperature of 101.3 the day of inoculation and no fever thereafter. The vena puncture control dog 7S was not febrile during the test period.

Little change in the differential leukocyte count, hemoglobin, and packed cell volume was observed.

The 3 dogs that had the polyethylene tube passed into their bronchi had positive serum CRP titers later in the test period. Five and 7 days after receiving mineral oil, dog 1S had a titer of 1:2. Dog 3S did not develop a CRP titer until 7 days after mineral oil administration. Control dog 5S had a positive serum CRP titer on the same days as dog 1S which had received mineral oil, and the strength of the agglutination reaction was stronger (Appendix, Table 1). The serums from vena puncture control dog 7S were not positive for CRP.

Postmortem examination of dogs 1S and 3S revealed atelectasis and emphysema at the ventral aspect of the apical and cardiac lobes of both lungs of both dogs. The atelectic areas were from 1.5 to 2.5 cm. in diameter. The emphysematous areas were immediately adjacent to the atelectic areas and of approximately the same size. The lungs of control dogs 5S and 7S did not develop these lesions. The trachea and major bronchi of all dogs were grossly normal.

Lung tissues from all dogs in the group were sectioned and stained for lipid content with Oil-Red-O. The sections from dogs 1S and 3S were positive for lipid, and the control dogs 5S and 7S were negative. Microscopic examination of the sections from the test animals revealed a large number of congested blood vessels and several vessels with edematous adventitia. Macrophages and an occasional giant cell filled the alveoli. The macrophages were filled with lipid droplets which stained red with Oil-Red-O. The alveolar walls were thickened by infiltrations of mononuclear cells and fibrosis. Lipid-laden macrophages also were found concentrated around the peribronchial blood and lymphatic vessels. Epithelization of the alveolar walls was observed in some areas. Atelectasis and adjacent emphysema were present. An occasional focal infiltration of neutrophils surrounding a bronchiole was seen in lung sections from control dog 5S. Atelectasis was found in some of the sections of lung from the vena puncture control dog 7S.

Group III—The test dogs 1X, 2X, 3B, and 5B were each inoculated intraperitoneally with a 10-ml. suspension containing 2.5 Gm. of talcum powder in physiologic saline. The control dog 3X was inoculated with 10 ml. of physiologic saline intraperitoneally, and dog 4X was the vena puncture control.

The test dogs receiving the talcum powder suspensions immediately developed signs of abdominal pain and became restless. Occasionally, one of the test dogs howled with apparent pain. After 5 minutes, these actions had stopped, and though these animals appeared lethargic, the early acute pain had subsided.

No such actions were displayed by dogs 3X and 4X. None of the dogs in this group exhibited any more clinical signs of abdominal pain in the days that followed.

A correlation of the total leukocyte count, CRP titer, and rectal temperature for the dogs in Group III is presented in Figs. 3 and 4.

Test dogs 1X, 2X, 3B, and 5B developed a marked leukocytosis 24 hours postinoculation. Neither of the 2 control dogs had a leukocytosis at this time. All of the test animals had an absolute neutrophilia, absolute lymphopenia, absolute eosinopenia, and regenerative shift to the left in the total leukocyte count at this time (Appendix, Table 1). By 3 days postinoculation, a shift to the right had occurred in the 4 dogs receiving talcum powder suspensions, and the leukocyte differential counts began to return to normal in the days that followed. A leukocytosis was present in test dog 1X the last day, and a shift to the right with hypersegmentation of neutrophils was recorded.

Control dog 3X, which received saline alone, developed a leukopenia 24 hours postinoculation and an absolute neutropenia, absolute lymphopenia, and absolute eosinopenia, with a relative neutrophilia (Appendix, Table 1). By the final day of the test period, this animal had a leukocytosis and shift to the right with hypersegmentation. The hemoglobin and packed cell volume levels on days 3, 5, and 7 were indicative of a borderline anemia. Dog 3X developed the only febrile reaction of the animals in this group 3 days postinoculation when the temperature recorded was 104.8 F. The vena puncture control animal 4X developed a

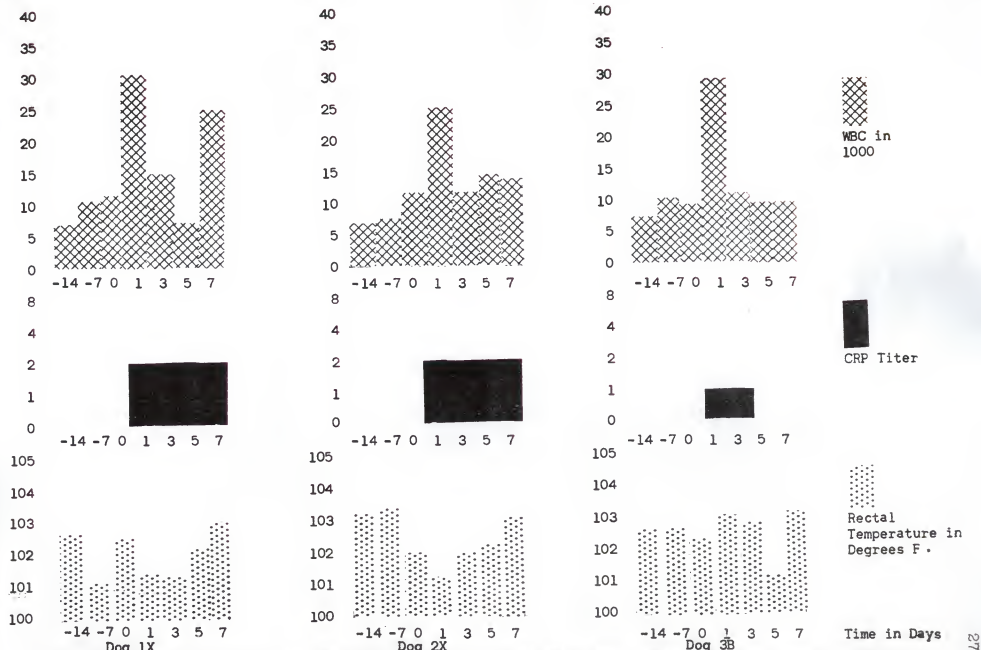


Fig. 3. A Correlation of Total White Cell Count, CRP Titer, and Rectal Temperature for Dogs in GROUP III.

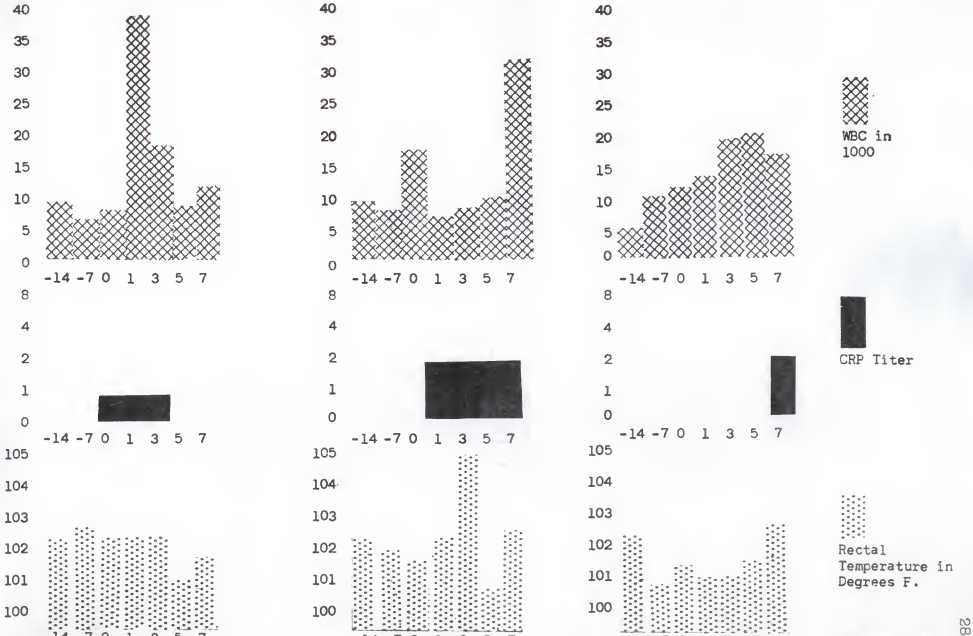


Fig. 4. A Correlation of Total White Cell Count, CRP Titer, and Rectal Temperature for Dogs in GROUP III (cont.)

leukocytosis 3 days after the inoculations, which persisted until the end of the test period. The relative leukocyte differential counts of dog 4X were within normal limits but the absolute values were slightly elevated.

Serums from all 4 dogs receiving talcum powder suspensions were positive for CRP 24 hours after inoculation. These results are graphically illustrated in Figs. 3 and 4 and tabulated in Appendix, Table 1. Test dog 5B had a CRP agglutination reaction of 4+ in the undiluted serum sample the day of inoculation. The control dog 3X developed a CRP reaction after receiving physiologic saline by intraperitoneal inoculation, which was as strong as those animals which had received talcum powder suspensions. The vena puncture control animal had a positive CRP serum the last day of the test period.

On several different days during the experimental period, the serum CRP reaction was the only test performed which indicated that an inflammation was present in the animal (Figs. 3 and 4). Five days postinoculation serum from test dog 1X had a CRP titer of 1:2 and a reaction of 2+, while the total and differential leukocyte counts and rectal temperature were within normal limits. Blood from test dog 2X had normal leukocyte counts as well as normal rectal temperature values 3 and 5 days postinoculation. Dog 2X's serum on those days had a CRP titer of 1:2, with a reaction strength of 3+. Three days after inoculation of test dog 3B, the CRP reaction of 3+ in the undiluted serum sample was the only test performed that indicated the presence of inflammation. The same was true of the CRP titer of 1:2, 1 and 5

days postinoculation, in the serums of control dog 3X.

A chronic peritonitis with extensive adhesions involving the omentum, small intestines, and parietal and visceral peritoneum was found on postmortem examination of the dogs that had received talcum powder suspensions. Approximately 100 ml. of cloudy ascitic fluid was present in each abdominal cavity. The entire distal half of the jejunum and all of the ileum were adhered to the omentum and the ventral abdominal wall in one large homogeneous mass. Portions of the intestines could not be separated from the omentum due to extensive adhesions. In dog 2X, the lower 1/4 of the spleen was covered with a thin layer of fibrin containing talcum powder deposits. Talcum powder was not detected grossly in the other test animals. The margins of all fibrosed areas were very hyperemic. Control dog 3X, which received 10 ml. of sterile physiologic saline intraperitoneally, had adhesions. A roughly elliptical area 8 cm. by 4 cm. of the omentum and ileum was adhered to the ventral abdominal wall, and the mass was separated with some difficulty. No gross lesions were seen in the vena puncture control dog 4X.

Microscopic sections from the inoculated dogs were examined and extensive proliferation of granulation tissue was observed. The serosa of the affected organs had undergone extensive fibrosis, and many small parallel branching capillaries were seen in the tissues. A diffuse infiltration of lymphocytes, plasma cells, and many macrophages was present. Most of the macrophages contained "sliver-like" crystals similar to the magnesium silicate crystals present in talcum powder. Similar crystals were found trapped in

the proliferating fibrous tissue. An occasional multinucleated giant cell was observed, some containing crystals. Fibrin observed in many areas appeared to be undergoing organization.

The reaction in control dog 3X was a similar granulomatous inflammation, but crystals were not observed and the organization was not as extensive. No microscopic lesions were observed in the vena puncture control dog 4X.

Group IV—This group consisted of 10 dogs and was part of a measles-canine distemper project being conducted simultaneously. Dogs 2M, 3M, 4M, 5M, 6M, 7M, and 9M were inoculated subcutaneously with canine-adapted human measles virus in tissue culture fluid. Control dogs 1M and 8M were inoculated with tissue culture fluid, and control dog 10M was not inoculated.

The data from Group IV animals are presented in Appendix Tables 1, 2, and 3, and graphically in Figs. 5, 6, and 7. All serum samples from this group had been frozen for 4 months before being tested for CRP, and all were negative when tested. These same dogs were given a challenge dose of canine distemper virus in Group V.

Group V—This group was composed of the dogs from Group IV immediately prior to and after intracranial challenge with virulent canine distemper virus one month after vaccination. Dogs 1M, 8M, and 10M were unvaccinated controls. Dog 7M had succumbed to a bacterial enteritis in the interim.

No clinical signs were observed in the dogs until 4 days postinoculation when the unvaccinated controls became lethargic and anorexic, and developed a reddening of the nasal mucosa and

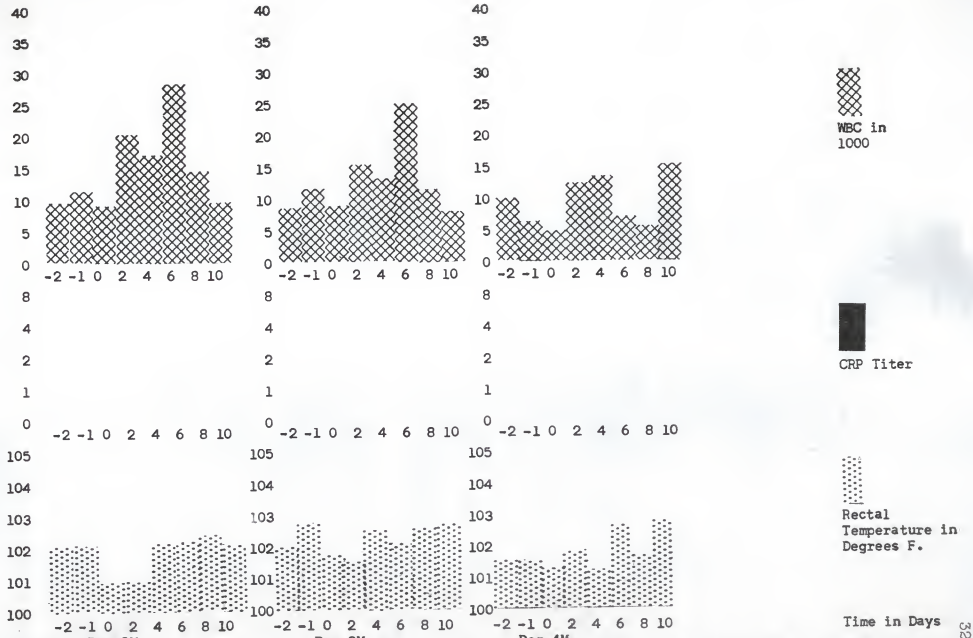


Fig. 5. A Correlation of Total White Cell Count, CRP Titer, and Rectal Temperature for Dogs in GROUP IV.

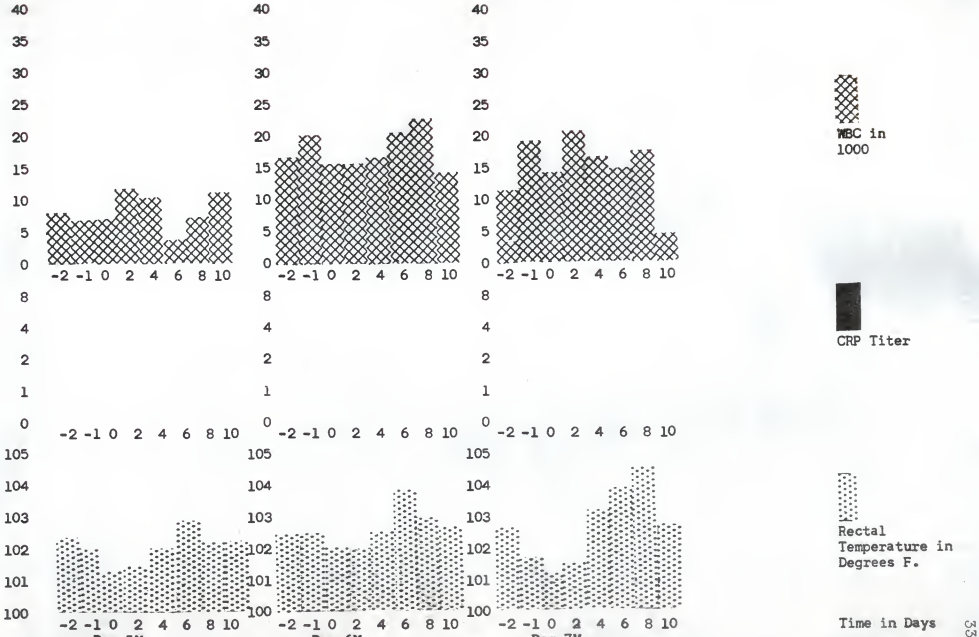


Fig. 6. A Correlation of Total White Cell Count, CRP Titer, and Rectal Temperature for Dogs in GROUP IV (cont.)

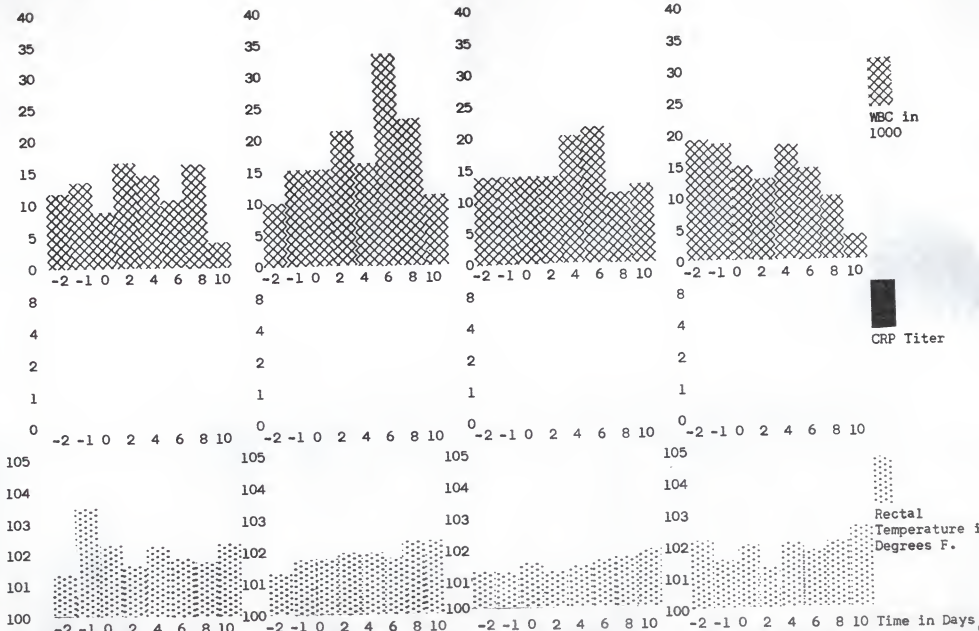


Fig. 7. A Correlation of Total White Cell Count, CRP Titer, and Rectal Temperature for Dogs in GROUP IV (cont.)

conjunctiva. In the days that followed, these dogs developed a purulent conjunctivitis and rhinitis, and became febrile. Encephalitic signs, including convulsions typical of canine distemper, were observed. The vaccinated dogs did not develop these signs.

A correlation of the total leukocyte count, CRP titer, and rectal temperature for the dogs in Group V is presented in Figs. 8, 9, and 10.

Vaccinated test-dog 2M developed a fever at 4 and 10 days after inoculation, and a leukocytosis, absolute neutrophilia, absolute lymphopenia, and absolute eosinopenis were present in the regenerative shift to the left at 10 days postinoculation. A severe bacterial enteritis developed in dog 2M at 9 and 10 days after challenge with distemper virus, and was successfully treated with oral and parenteral antibiotics on days 10 and 11. None of the other vaccinated test dogs developed febrile reactions, but test dog 4M had a leukocytosis with absolute increases in the neutrophils, lymphocytes, and eosinophils, and a regenerative shift to the left in the differential leukocyte count 10 days postinoculation. Dog 4M did not develop symptoms of disease. Minor changes in the differential leukocyte counts were recorded for the other test animals.

The unvaccinated control dogs all developed signs of canine distemper, and dogs 1M and 8M died. Dog 10M was euthanatized and exsanguinated as a part of the distemper project. Distemper-infected control dog 1M had a diphasic temperature variation

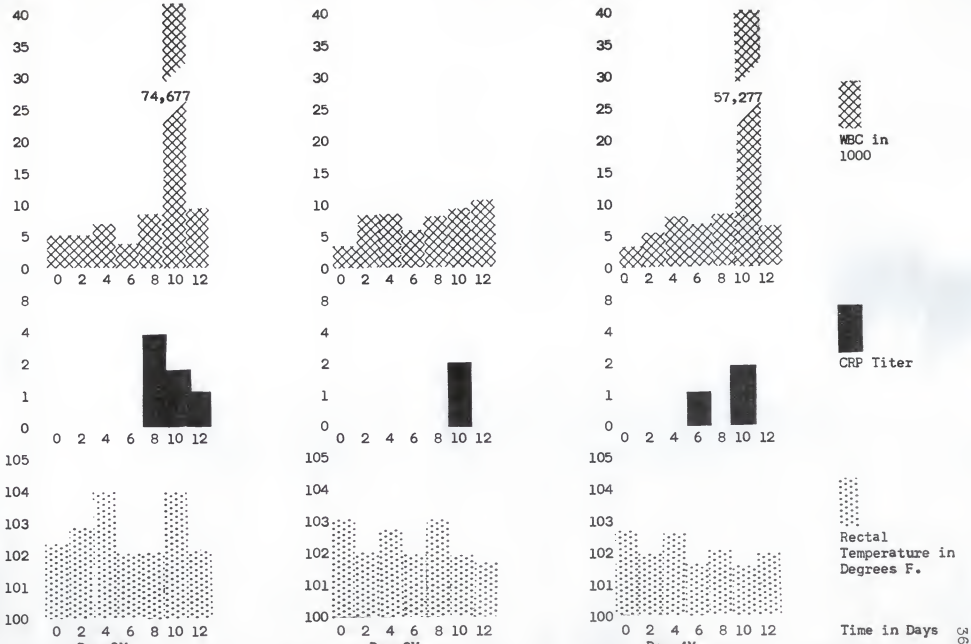


Fig. 8. A Correlation of Total White Cell Count, CRP Titer, and Rectal Temperature for Dogs in GROUP V.

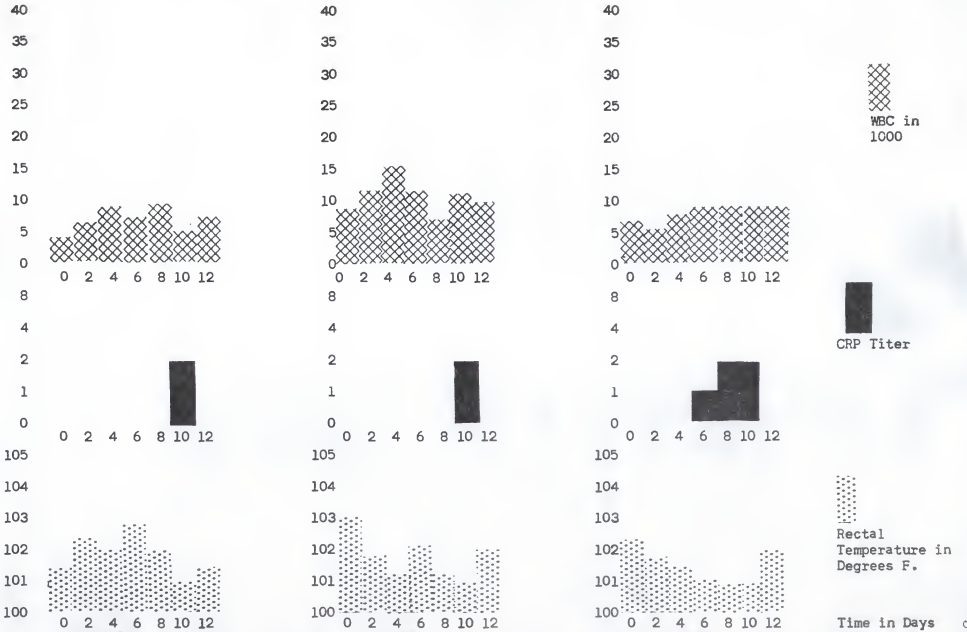
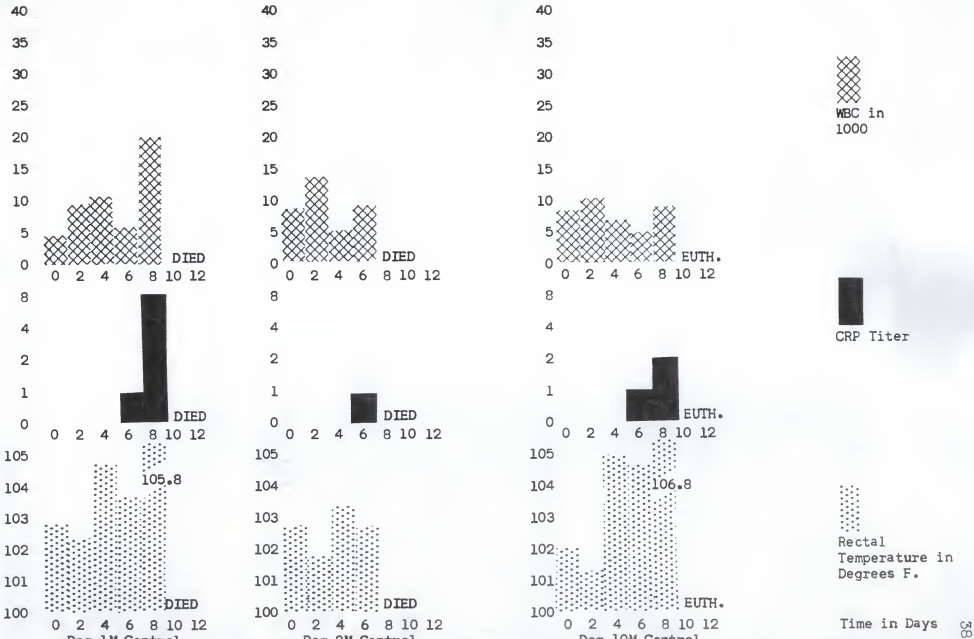


Fig. 9. A Correlation of Total White Cell Count, CRP Titer, and Rectal Temperature for Dogs in GROUP V (cont.)



Dog 8M Control

Fig. 10. A Correlation of Total White Cell Count, CRP Titer, and Rectal Temperature for Dogs in GROUP V (cont.)

between 4 and 8 days postinoculation, with a leukopenia on day 6 and a leukocytosis on day 8. An early regenerative shift to the left and later a shift to the right were recorded for dog 1M. Control dog 8M developed a leukopenia with an absolute neutropenia, absolute lymphopenia, and absolute eosinopenia 4 days after inoculation. The total leukocyte count was within normal limits 6 days after inoculation, and the absolute values of neutrophils and lymphocytes were only slightly lower than normal. Control dog 10M developed a leukopenia, absolute neutropenia, absolute lymphopenia, and absolute eosinopenia 6 days after inoculation. A shift to the right and absolute neutrophil and lymphocyte values approximating normal levels were recorded 8 days after inoculation.

Every dog in this group developed a positive CRP reaction sometime during the experimental period. The earliest reactions were obtained 6 days after inoculation of the distemper challenge virus. The last reaction to occur developed 10 days after inoculation. The highest titer obtained was 1:8 with a 2+ reaction in the unvaccinated control dog 1M. The lowest serum CRP value occurred in unvaccinated control dog 8M which died of distemper the next day. The vaccinated dogs developed as high CRP titers as the unvaccinated distemper controls. With the exception of vaccinated dog 2M, the vaccinated animals had serums that were negative for CRP by the end of the test period, while positive CRP serums persisted until death in all three control animals infected with distemper.

Group VI—This group consisted of 7 dogs. Dogs 2MM, 4MM, 6MM, and 9MM were inoculated with 10 ml. of a hemolytic strain of E. coli. Control dogs 3MM and 5MM received 10 ml. of nutrient broth by parenteral inoculation and dog 5X was the vena puncture control dog.

No immediate reaction was observed in any of the inoculated dogs. The 3 control animals did not manifest clinical signs during the experimental period. Twenty-four hours after the intraperitoneal inoculation of E. coli, dogs 4MM and 6MM were lethargic, and abdominal palpation elicited clinical signs of pain. Dogs 2MM and 9MM, which were inoculated subcutaneously with E. coli organisms, did not manifest any clinical signs at this time and appeared normal. Seventy-two hours after inoculation, animals 4MM and 6MM appeared clinically normal, and palpation of the abdomen did not elicit signs of pain. From this time on, dogs 4MM and 6MM were clinically normal. By 72 hours postinoculation, dogs 2MM and 9MM had developed painful swellings over the shoulder at the inoculation sites and were lethargic. Six days after inoculation, the swelling over the shoulder of dog 9MM ruptured and discharged a purulent exudate. Dog 2MM, at this time, had a subcutaneous swelling extending ventrally over the left ribs to a point near the sternum. Seven days postinoculation, this swelling ruptured near the sternum and discharged a purulent exudate.

A correlation of the total leukocyte count, CRP titer, and rectal temperature for dogs in Group VI is presented in Figs. 11 and 12.

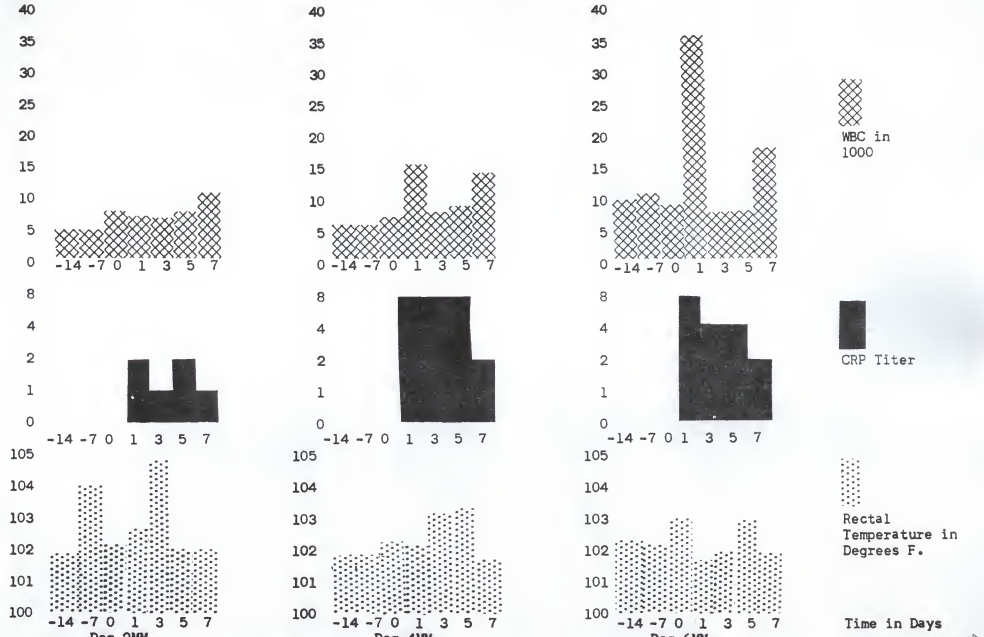


Fig. 11. A Correlation of Total White Cell Count, CRP Titer, and Rectal Temperature for Dogs in GROUP VI.

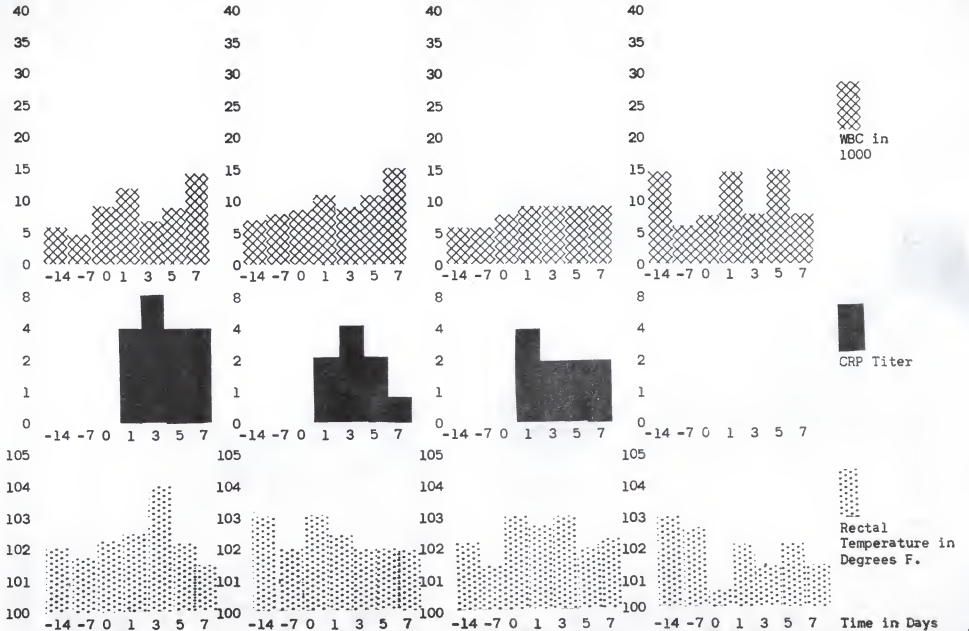


Fig. 12. A Correlation of Total White Cell Count, CRP Titer, and Rectal Temperature for Dogs in GROUP VI (cont.)

Dog 6MM developed the only pronounced leukocytosis and had a high leukocyte count 1 and 7 days after inoculation. The only marked postinoculation febrile reaction occurred in dogs 2MM and 9MM 3 days postinoculation. All inoculated dogs developed a shift to the left and an absolute neutrophilia following inoculation (Appendix, Table 1). The vena puncture control dog, 5X, developed a slight leukocytosis and a regenerative shift to the left with an absolute neutrophilia 5 days after the other animals had been inoculated.

Serums from this group developed the largest number of high CRP titers of any of the experimental groups. All of the inoculated dogs had serums positive for CRP, with vena puncture control dog 5X having the only CRP negative serums. All of the inoculated dogs had positive CRP serums 24 hours after inoculation, and the serums were positive in these dogs until the end of the experimental period. Control dogs 3MM and 5MM, which were inoculated with sterile nutrient broth, developed CRP titers almost as high as those animals receiving E. coli inoculations, and as high as the titers obtained in serums from test animal 2MM.

On several days during the experimental period, serum CRP was detected before changes in the total leukocyte count or rectal temperature indicated the presence of an inflammatory process. On postinoculation days 1, 5, and 7, dog 2MM had total leukocyte counts and rectal temperature values within normal limits, but the serum CRP titer on these days indicated that an inflammation was present. The serum CRP titer was present 24 hours postinoculation; a full day before the animal became febrile. Dog 9MM had

CRP positive serum 1 day postinoculation, which was 24 hours before changes in the total leukocyte count or rectal temperature indicated the presence of inflammation. The CRP positive serums from dog 9MM on postinoculation days 5 and 7 were indications of inflammation even though the total leukocyte count and rectal temperature were considered normal. This also was true throughout the postinoculation period for control dogs 3MM and 5MM that had received inoculations of nutrient broth. The appearance of serum CRP did correspond favorably with the appearance of immature forms of neutrophils in the differential counts performed on blood taken from this group during the experimental period (Appendix, Table 1).

Postmortem examination of test dogs 2MM and 9MM revealed a roughly circular area of 15-cm. diameter, in the subcutis at the inoculation site, that was filled with purulent exudate, and surrounded by edematous subcutaneous tissue with a hyperemic margin. In dog 2MM, a fistulous tract filled with purulent exudate extended down over the left thorax to an opening near the sternum. Test dogs 4MM and 6MM had extensive abdominal adhesions involving the omentum, small intestines, and parietal peritoneum of the ventral abdomen. There was no involvement of other abdominal organs, and no ascites was present. The involved intestine and omentum in these dogs formed a mass, approximately 10 cm. in diameter, which was firmly attached to the ventral abdominal wall. Control dog 3MM had a small circular area of subcutaneous edema 2.5 cm. in diameter. The omentum of control dog 5MM was adhered to the ventral abdominal wall, but the

intestines were not involved. The fibrosed area measured 6.2 cm. in diameter. No grossly inflamed tissues were observed in vena puncture control dog 5X.

Microscopic examination of the inflamed subcutaneous tissues from dogs 2MM and 9MM revealed the formation of early granulation tissue surrounding a purulent necrotic area. The margin of the necrotic area was filled with macrophages, lymphocytes, and plasma cells. Immediately surrounding this area, a fibrin matrix was present which was infiltrated with large numbers of fibroblasts. Outside the fibrosing area, numerous congested blood vessels were found. A diffuse infiltration of lymphocytes and plasma cells surrounded an area filled with pink staining serous fluid in the hematoxylin and eosin stained sections from control dog 3MM which received nutrient broth subcutaneously.

Sections from the inflamed tissues of dogs 4MM and 6MM had extensive proliferations of granulation tissue when examined microscopically. The intestinal serosa had undergone extensive fibrosis. Many small congested blood vessels were seen penetrating the fibrous tissue. A diffuse infiltration of lymphocytes, plasma cells, and macrophages was present. Pools of fibrin filled with fibroblasts were seen. The histopathologic picture seen in control dog 5MM was similar.

All inoculation sites of the dogs in this group were examined for the presence of bacteria. The subcutaneous tissue and abdominal cavity of the vena puncture control dog 5X also was examined. Coagulase positive Staphylococcus aureus and hemolytic E. coli were isolated from the subcutaneous lesions of dogs 2MM and 9MM.

No bacteria were isolated from the test dogs 4MM and 6MM that were inoculated intraperitoneally. No bacteria were isolated from the 2 control dogs 3MM and 5MM that received nutrient broth or from vena puncture control dog 5X.

Serum Electrophoretic Analysis

After CRP testing, each "frozen" serum sample and determining the stability of the CRP fraction at -20 C., each serum sample was allowed to thaw at room temperature and was submitted to paper electrophoresis. A summary of the relative per cent of each serum fraction and CRP endpoint titer for all of the serums is presented in Appendix, Table 2.

CRP Stability

The CRP agglutination reaction was performed on all "fresh," "refrigerated," and "frozen" serums as previously described. A summary of the results is presented in Appendix, Table 3. Although the strength of the agglutination reaction occasionally decreased in the frozen samples, the changes in the CRP endpoint titers were very few. All serums from Group IV were negative after storage at -20 C. for 4 months. C-reactive protein determinations were not performed on "fresh" and "refrigerated" serums from this group.

After the CRP stability at -20 C. had been determined, 10 CRP positive and 10 CRP negative serums were selected from the 283 serum samples for heat stability determinations. The various samples had been frozen for 1 to 2 months at that time. The

serums were again tested for CRP, and the endpoints were found to be the same as those recorded earlier. The 20 serums and their twofold dilutions were placed in a water bath at 70 C. for 30 minutes. At the end of this time, the CRP tests were again conducted. All of the serums and dilutions were negative when tested for CRP after the heat treatment.

Analysis of Data

In order to detect and emphasize trends in the data, means of the CRP titers, rectal temperatures, total leukocyte counts, and serum fraction relative per cent values were calculated. These means are presented in Tables 2, 3, 4, and 5.

Table 2 is a summary of the means of the rectal temperatures, total leukocyte counts, and serum fraction relative per cent values at each CRP titer by experimental groups. The relative per cent of the beta serum fraction most consistently increased with increasing CRP levels in each group, while the variability of the means of the relative per cent values of the other serum fractions was quite marked within and between groups. In 2 instances, the beta serum fraction value decreased with increasing CRP titer; in Group V, at a CRP titer of 1:4, and in Group VI, at a CRP titer of 1:2. Group IV means were included as additional information on the same dogs in Group V.

Increases in the mean CRP values recorded in Table 2 were consistent with increases in the mean rectal temperature and total leukocyte count with some notable exceptions. The CRP values in Group II increased with the mean total leukocyte count values,

TABLE 2. A Summary, by Experimental Groups, of Means of the Temperature, Total Leukocyte Count, and Canine Serum Fractions at Each C-Reactive Protein Titer

			Group I				
			%	% Globulins			
CRP*	Temp.**	WBC+	Albumin	Alpha-1	Alpha-2	Beta	Gamma
0	101.9	9357	35.7	8.6	15.7	10.6	29.4
1	101.8	7133	34.0	9.2	20.0	12.5	24.4
2	104.8	14282	29.9	10.5	20.9	14.4	24.4
4	.....	.....	....	....	....	....	....
8	.....	.....	....	....	....	....	....
			Group II				
0	102.5	9367	40.3	10.5	20.3	8.2	20.6
1	.....	.....	....	....	....	....	....
2	102.5	13780	44.8	6.9	17.3	10.4	20.6
4	.....	.....	....	...	....	....	....
8	.....	.....	....	...	....	....	....
			Group III				
0	102.0	10501	32.1	7.7	20.0	13.4	26.9
1	102.6	20968	33.5	4.8	19.5	13.7	28.5
2	102.2	16601	31.6	5.5	20.2	16.6	26.2
4	.....	.....	....	...	....	....	....
8	.....	.....	....	...	....	....	....
			Group IV++				
0	102.0	13866	54.2	9.3	11.5	8.6	16.3
1	.....	.....	....	...	....	...	....
2	.....	.....	....	...	....	...	....
4	.....	.....	....	...	....	...	....
8	.....	.....	....	...	....	...	....
			Group V				
0	102.4	7550	48.0	9.5	18.5	7.9	16.1
1	102.7	7031	46.4	7.8	22.9	7.9	15.1
2	102.3	22609	48.1	7.1	20.3	8.2	16.3
4	102.0	6500	50.2	5.2	23.4	7.1	14.1
8	105.8	20200	47.6	5.9	23.5	9.5	13.6
			Group VI				
0	102.2	8501	41.1	8.8	17.4	11.6	21.0
1	102.9	11620	40.9	6.4	18.6	11.7	22.4
2	102.2	11026	45.5	7.2	15.4	11.2	20.7
4	102.3	10530	42.8	8.5	16.7	12.2	19.7
8	102.9	15629	36.3	8.1	20.1	15.4	20.1

* C-reactive protein titer.
** Mean of the rectal temperatures in degrees Fahrenheit.
\+ Mean of the total leukocyte counts.
++ Group IV had no serums that were positive for CRP.

TABLE 3. A Summary, by Experimental Groups, of Means of the C-Reactive Protein Titer, Temperature, Total Leukocyte Count, and Serum Fractions, During the Preinoculation and Postinoculation Periods of the Test vs Control Dogs

Dogs	Time*	CRP**	Temp.+	WBC++	Albu-min	% Globulins Alpha -1	Alpha -2	Beta	Gamma
Group I									
Test	Pre.	0.16	102.4	8404	36.7	7.6	18.3	9.9	27.5
	Post.	0.50	102.2	12798	34.5	8.4	19.1	13.3	24.8
Control	Pre.	0.00	102.2	9499	37.4	13.0	12.7	11.1	25.8
	Post.	0.00	102.0	9379	35.1	11.4	10.6	10.5	32.4
V.P. Control#	....	0.00	102.1	7257	33.8	7.0	15.8	9.3	34.1
Group II									
Test	Pre.	0.00	102.4	8446	43.9	10.7	18.1	8.0	19.2
	Post.	0.75	102.5	11700	43.7	9.7	20.1	8.5	18.1
Control	Pre.	0.00	102.6	9189	37.5	8.4	22.3	7.7	24.1
	Post.	1.00	102.8	13673	37.9	7.0	20.9	10.7	23.4
V.P. Control	....	0.00	102.4	8258	39.3	11.6	19.0	8.5	21.5
Group III									
Test	Pre.	0.08	102.5	8870	35.2	6.9	19.7	13.3	24.8
	Post.	1.37	102.1	9221	31.5	6.5	23.6	13.5	24.9
Control	Pre.	0.00	102.2	11809	29.9	7.3	18.3	15.0	29.5
	Post.	2.00	102.7	14110	24.1	5.3	14.4	23.9	32.3
V.P. Control	....	0.28	101.4	13267	32.3	7.2	16.4	14.1	29.9

* Relative to day of inoculation.

** Mean of the C-reactive protein titers.

\+ Mean of the rectal temperatures in degrees Fahrenheit.

++ Mean of the total leukocyte counts.

\# Vena puncture control dogs.

TABLE 3 (concl.)

Dogs	Time	CRP	Temp.	WBC	Albumin	% Globulins Alpha -1	Alpha -2	Beta	Gamma
Group IV##									
Test	Pre.	0.00	101.9	11277	56.5	8.5	11.5	8.5	15.0
	Post.	0.00	102.3	13994	53.2	9.0	12.6	8.5	16.7
Control	Pre.	0.00	101.6	15034	55.5	9.7	10.3	9.1	15.4
	Post.	0.00	101.9	16559	51.7	9.9	11.5	8.3	18.6
Group V##									
Test	....	0.50	102.1	10196	48.7	9.1	18.8	7.8	15.6
Control	....	0.92	103.6	8824	46.2	8.3	21.2	8.2	16.1
Group VI									
Test	Pre.	0.00	102.3	7695	43.5	8.6	17.5	11.1	19.3
	Post.	4.37	103.2	12530	37.1	7.8	19.4	14.1	21.6
Control	Pre.	0.00	102.5	7724	47.0	8.7	17.9	9.5	16.8
	Post.	2.37	102.4	10684	52.4	7.5	12.7	9.2	18.3
V.P. Control	....	0.00	101.9	10549	31.8	9.3	16.8	14.4	27.7

Groups IV and V did not have vena puncture control dogs.

TABLE 4. A Summary of Means of the Temperature, Total Leukocyte Count, and Serum Fractions, of All Experimental Dogs, at Each C-Reactive Protein Titer

CRP*	Temp.**	WBC+	% Albumin	% Globulins Alpha-1	Alpha-2	Beta	Gamma
0	102.2	9055	41.9	9.1	17.2	10.1	21.7
1	102.5	11688	38.7	7.1	20.3	11.5	22.5
2	102.8	15660	40.0	7.4	18.8	12.2	21.6
4	102.2	8515	46.5	6.9	20.1	9.7	16.9
8	104.4	17915	42.0	7.0	21.7	12.5	16.9

* C-reactive protein titer.
** Mean of the rectal temperatures in degrees Fahrenheit.
\+ Mean of the total leukocyte counts.

TABLE 5. A Summary of Means of the C-Reactive Protein Titer, Temperature, Total Leukocyte Count, and Serum Fractions, During the Preinoculation and Postinoculation Periods of the Test vs Control Dogs

Dogs	Time*	CRP**	Temp.+	WBC++	Albumin	% Globulins Alpha -1	Alpha -2	Beta	Gamma
Test#	Pre.	0.05	102.3	8938	43.2	8.5	17.0	10.2	21.2
	Post.	1.31	102.7	11511	41.0	8.3	19.3	11.0	20.4
Control##	Pre.	0.00	102.2	10651	41.5	9.4	16.3	10.5	22.3
	Post.	0.97	102.3	12428	41.7	8.4	14.8	11.7	23.4
V.P. Control###	....	0.07	101.9	9631	34.5	8.7	16.8	11.5	28.5

* Relative to day of inoculation.
** Mean of the C-reactive protein titers.
\+ Mean of the rectal temperatures in degrees Fahrenheit.
\++ Mean of the total leukocyte counts.
\# Test animals in Groups IV and V were unvaccinated distemper control dogs.
\## Control animals in Groups IV and V were vaccinated distemper test dogs.
\### Does not include Groups IV and V which had no vena puncture control dogs.

but not with the mean temperatures. The mean values for temperature and total leukocyte count in Group V did not increase with serum CRP titers of 1:2 and 1:4. These values did not correspond with the other values obtained in Group V, but this was reasonable when one considered that the dogs infected with canine distemper developed leukopenias along with CRP positive serums. The bacterial enteritis which developed a CRP titer of 1:2 in test dog 2M (Fig. 8) produced a total leukocyte count of 74,677 which was responsible for the increase in the mean of the total leukocyte counts at the CRP mean of 1:2 for Group V.

Table 3 is a summary of the means of the CRP titers, rectal temperatures, total leukocyte counts, and serum fraction relative per cent values of the test and control dogs of each group before and after inoculation. The CRP positive serums consistently had higher postinoculation than preinoculation means in every group. A series of preinoculation serums was not taken immediately prior to inoculation in Group V, although the serums in Group IV were from these same dogs one month earlier. The CRP means increased with the means of the total leukocyte count in every group except Group V. In Group V, the leukopenia resulting from canine distemper was apparent and corresponded to the increased mean CRP titer recorded. With two exceptions, the CRP mean values increased with the means of the rectal temperatures. The exceptions were the preinoculation and postinoculation mean values for the test dogs in Group III and the preinoculation and postinoculation mean values for the control dogs in Group VI. The CRP mean values increased with increasing beta serum fraction means with one

exception. The mean CRP value of the control dogs in Group VI increased from 0.00 to 2.37 while the beta serum fraction per cent dropped from 9.5 to 9.2.

Table 4 is a summary of the means of the rectal temperatures, total leukocyte counts, and per cent of each serum fraction at each CRP titer for all 283 blood samples analyzed. An increased mean CRP value corresponded to an increased mean value for the rectal temperature, total leukocyte count, and per cent beta globulin with the exception of the CRP titer of 1:4.

Table 5 is a summary of means of the CRP titers, rectal temperatures, total leukocyte counts, and per cent of each serum fraction during the preinoculation and postinoculation period of the test and control animals. Without exception, an increase in the mean CRP value was consistent with an increase in the mean values of the temperature, total leukocyte count, and per cent beta serum globulin.

DISCUSSION

In CRP testing serums from the first experimental groups, the known CRP positive human control serum, in two instances, did not react. Investigation revealed that contaminants present on the ruled glass plate apparently were responsible for inhibition of the agglutination reaction. When the glass plate was thoroughly washed with triple distilled water, the CRP positive human control serum consistently reacted with the latex reagent, and the results obtained from the canine serums could be demonstrated repeatedly. If the glass plate was washed with tap water, the

results of the agglutination reactions were variable when repeated on the same serums.

A great deal of individual variation in the positive CRP reactions was observed. This concurs with the results obtained by Ash (1933), who used the pneumococcal C-polysaccharide precipitation reaction in clinical studies on human serums, Wood (1953) in his studies on CxRP response in rabbits, and with the findings of Hedlund (1961) in his work with CSR in rabbits. It is possible that the variable CRP reactions noted in canine serum may have been partially due to the lack of an antiserum specific for the canine CRP-like fraction. An example of the variability encountered in this work is graphically presented by the unvaccinated control dogs 1M and 8M, both of which died of experimentally produced canine distemper (Fig. 10).

In examining the data presented graphically in Figs. 1 to 12 and the tabulated data in Appendix, Table 1, it was noted that on some days the presence of a CRP titer was the only analysis performed which indicated an inflammatory reaction. In Group III, test dogs 1X, 2X, and 3B had positive CRP serums on days when the total and differential leukocyte counts and rectal temperatures were considered normal. On certain days, dogs 2MM, 3MM, 5MM, and 9MM, in Group VI, had CRP positive serums when the total leukocyte counts and rectal temperatures were considered normal. The occurrence of immature neutrophils corresponded with the appearance of CRP in Group VI, however, and confirmed the presence of an inflammation during the postinoculation period. The postmortem lesions present 7 days after inoculation confirmed the presence

of inflammation during the postinoculation period in all the test dogs except those in Groups IV and V which were not necropsied.

A positive CRP reaction occurred as early as 24 hours post-inoculation in Groups I, III, and VI. Roantree and Rantz (1955) believed that the early appearance of CRP in human serums, during the course of inflammation, was one of the more important properties of human CRP. Anderson and McCarty (1950) observed transitory serum CRP titers in human patients who recovered from acute attacks of rheumatic fever, and persistent serum CRP titers in patients who did not recover from the disease. Wood (1958) noted transitory serum CRP titers in certain acute forms of Hodgkin's disease, and low but persistent titers in humans who developed the chronic form. The dogs in Group VI that survived the challenge with virulent canine distemper virus, developed transitory serum CRP titers, while those which succumbed to the infection had CRP positive serums until death.

Some of the control dogs in Groups II, III, V, and VI developed positive CRP serums. The unvaccinated control dogs in Group V were controls for the measles-distemper project and were more properly the "test" animals in relation to the CRP study. The possibility that bacteria were introduced into some of the control dogs in Groups II, III, and VI cannot be discounted. No gross lesions were detected in control dog 5S in Group II, and microscopic examination of the lung tissues revealed only an occasional peribronchial infiltration of neutrophils which could have resulted from the introduction of bacteria into the respiratory passages by passage of the polyethylene tube.

Hedlund (1961) was able to produce CSR in the rabbit by inoculating a wide variety of substances. He found that 5 ml. of physiologic saline inoculated intraperitoneally into rabbits produced positive CSR titers of 1:8 in 4 out of 9 of the animals. The positive CRP titers obtained from control dog 3X in Group III suggested that the canine responds in a similar manner. He noted that therapeutic amino acid and peptone solutions inoculated intraperitoneally into rabbits produced positive CSR titers in 11 of 16 of the animals. The occurrence of positive CRP serums in Group VI control dogs 3MM and 5MM, that received nutrient broth subcutaneously and intraperitoneally, respectively, suggested that the reaction might also occur in the canine. No difference in CRP response between those dogs in Group VI that received intraperitoneal inoculations of bacteria and those that received subcutaneous inoculations was observed. These results concurred with the early findings of Abernathy (1937) in his work with monkeys, and with the later work of Hedlund (1961) who worked with laboratory animals. Hedlund also found that live or dead bacteria stimulated the formation of more CSR in the rabbit than did many foreign substances. Table 3 indicates that higher CRP levels were obtained from the E. coli inoculations in Group VI than were obtained from any of the other groups.

Three of the 66 positive CRP reactions occurred at unexpected times: the CRP reaction on the day of inoculation of test dog 1B in Group I (Fig. 1); the CRP reaction on the day of inoculation of test dog 5B in Group III (Fig. 4); and the CRP reaction 7 days postinoculation of vena puncture control dog 4X in Group III

(Fig. 4). The CRP negative serum from test dog 4M in Group V, collected 8 days after inoculation, was an unexpected CRP negative serum sample (Fig. 8).

The positive CRP reaction in dog 1B on the day of inoculation occurred in the undiluted serum sample, and had an agglutination reaction strength of 1+ (Appendix, Table 1). The CRP reaction which occurred the day of inoculation in test dog 5B in Group III also occurred in the undiluted serum sample, but had a strong agglutination reaction of 4+. Two possible explanations for these serum reactions exist. Since a period of 7 days existed between the preinoculation blood withdrawal and the collection of blood on the day of inoculation, the animals could have been traumatized in the interim. The dogs were housed in stalls by litters, and frequently were observed scrapping and fighting. It is possible that they could have been traumatized in this manner. The other possibility is that the reactions were true "non-specific" reactions occurring as a result of the use of a cross-reacting reagent, specific for human CRP and not specific for the analogous canine serum fraction.

The positive CRP reaction in vena puncture control dog 4X, in Group III, 7 days postinoculation could have occurred as a result of trauma induced by a series of poor vena punctures. Indeed, this was the reason for including a vena puncture control animal in each experimental group.

An explanation for the failure of the 8-day postinoculation serum from test dog 4M in Group V to react is not offered. Hedlund (1961) encountered the same phenomenon with CxRP in

frostbite experiments conducted on 8 rabbits, and he offered no explanation.

The data from Tables 2, 3, 4, and 5 indicate that an increase in serum CRP is consistent with an increase in rectal temperature and total leukocyte count. There were some notable exceptions but it was felt that the relationship was remarkable when one considered that the reagent used in the CRP reaction was not specific for the canine CRP-like serum fraction. There was some indication that canine CRP is, in some way, associated with the beta serum globulin fraction. Wood et al. (1954) came to similar conclusions as a result of studies on human CRP. That human CRP is composed of more than one fraction has since been well established by Libretti et al. (1955), Hedlund (1958), and Fishel et al. (1960). Further work with canine CRP might produce similar results.

The stability of the canine CRP fraction when stored at -20 C. was indicated by the results presented in Appendix, Table 3. Löfström (1943) demonstrated the stability of human CRP when stored for years at -20 C. The canine CRP fraction was deactivated in 10 serum samples and twofold dilutions after exposure to 70 C. for 30 minutes. Abernathy and Avery (1941) observed deactivation of human CRP at this temperature exposure, and felt that the results indicated the protein-like quality of the fraction since temperatures in excess of 65 C. denature most proteins.

CONCLUSIONS

A non-specific serum fraction was present in the serums of dogs during some experimentally induced inflammations. The canine serum fraction's stability at -20 C., lability at 70 C., and occurrence during inflammation are properties analogous to human CRP.

Considerable individual variability in CRP response was observed and partially explained as having resulted from the use of a cross-reacting agglutinating reagent, specific for human CRP, for the detection of the analogous canine serum fraction. The appearance during inflammation of the canine serum fraction suggested that an antiserum specific for the canine acute phase serum fraction could be an aid to prognosis in the practice of veterinary medicine, and that further work should be done to develop and evaluate such a reagent.

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APPENDIX

TABLE 1. A Summary of Results of the Hematologic and C-Reactive Protein Examinations in Six Groups of Experimental Dogs

Dog No.	Day	Temp.*	CRP**	WBC+	Differential leukocyte count++ Juv.	Band	Seg.	Lym.	Eos.	Mon.	Hb.#	PCV##
GROUP I												
	-14	102.8	0(0)	10404	0	0	28	42	27	3	20.0	33
	- 7	102.8	0(0)	9039	0	3	56	30	7	4	16.0	40
	0	101.8	1(1)	7133	0	0	59	37	3	1	13.2	34
1B	1	104.8	2(1)	16038	0	2	68	21	5	4	14.4	43
	3	101.0	0(0)	19330	0	0	69	26	2	3	15.1	45
	5	101.8	0(0)	8697	0	0	59	37	4	0	17.2	40
	7	101.0	0(0)	11173	0	8	52	26	13	1	14.4	41
	-14	102.6	0(0)	10962	0	0	55	31	10	3	19.6	46
	- 7	102.7	0(0)	7640	0	0	42	50	8	0	16.0	44
	0	101.6	0(0)	5247	0	2	51	37	6	4	14.2	39
4B	1	104.8	2(1)	12525	0	1	74	18	4	0	16.5	50
	3	101.2	0(0)	11222	0	0	68	30	1	1	16.4	49
	5	101.8	0(0)	10443	0	2	64	30	3	1	16.0	45
	7	101.2	0(0)	12956	0	10	51	30	4	5	15.6	45
	-14	102.8	0(0)	13850	0	3	60	22	14	1	15.2	40
	- 7	102.5	0(0)	9144	0	0	33	67	0	0	17.5	40
	0	101.2	0(0)	5503	0	0	38	52	9	1	13.2	36
2B C.°	1	102.0	0(0)	6614	0	12	34	34	16	4	14.8	40
	3	102.8	0(0)	13900	0	3	58	27	9	3	14.0	40
	5	102.5	0(0)	10500	0	2	64	30	3	1	16.5	45
	7	101.0	0(0)	6500	0	2	46	31	16	5	15.0	40
	-14	102.4	0(0)	5889	3	6	29	41	16	5	13.6	33
	- 7	102.4	0(0)	8044	0	3	55	35	4	3	16.0	33
	0	102.2	0(0)	6088	0	0	44	46	4	6	13.9	35
6B V.P.C.°°	1	101.2	0(0)	8990	0	0	55	35	6	4	16.0	45
	3	102.0	0(0)	8728	0	0	51	39	6	4	16.0	43
	5	102.2	0(0)	8382	0	0	64	32	4	0	16.3	40
	7	102.0	0(0)	4678	0	2	64	30	4	0	14.6	38

* Rectal temperature in degrees Fahrenheit.

** The first number represents the C-reactive protein titer, and the number within the parentheses represents the strength of the agglutination reaction at that dilution.

\+ Total leukocyte count.

++ The abbreviations for the types of leukocytes counted are: Juv. = Juvenile neutrophil, Band = Band neutrophil, Seg. = Segmented neutrophil, Lym. = Lymphocyte, Eos. = Eosinophil, and Mon. = Monocyte.

\# Blood hemoglobin in Gm./100 cc.

Packed cell volume in per cent.

° Control dog.

°° Vena puncture control dog.

TABLE 1 (cont.)

Dog No.	Day	Temp.	CRP	WBC	Differential leukocyte count						Hb.	PCV
					Juv.	Band	Seg.	Lym.	Eos.	Mon.		
				GROUP II								
	-14	102.8	0(0)	4784	0	1	47	46	6	0	11.5	30
	- 7	102.0	0(0)	10637	0	1	47	45	6	0	16.0	45
	0	103.2	0(0)	5226	0	2	52	30	12	4	16.6	45
1S	1	103.2	0(0)	7777	0	0	53	37	5	5	13.4	44
	3	103.2	0(0)	12234	0	0	58	30	8	4	16.6	43
	5	103.2	2(2)	17363	0	0	60	36	2	2	16.6	43
	7	102.1	2(1)	9879	0	0	60	34	5	1	15.2	40
	-14	103.0	0(0)	6203	0	2	44	43	6	5	10.0	30
	- 7	101.8	0(0)	14832	0	0	42	50	8	0	14.8	42
	0	101.8	0(0)	8996	0	1	52	34	7	6	16.0	43
3S	1	102.0	0(0)	7339	0	0	45	46	4	5	16.0	42
	3	102.5	0(0)	15574	0	0	52	42	2	4	15.2	43
	5	102.0	0(0)	13872	0	0	53	42	1	4	16.4	42
	7	101.2	2(4)	9559	0	0	56	36	8	0	15.6	41
	-14	102.4	0(0)	5704	0	1	35	48	13	3	13.6	39
	- 7	104.2	0(0)	11894	0	2	64	25	8	1	14.0	40
	0	101.3	0(0)	9972	0	0	53	38	6	3	16.6	47
5S C.	1	102.8	0(0)	8455	0	0	50	33	2	5	16.0	45
	3	102.7	0(0)	14138	0	0	45	41	13	1	17.0	46
	5	102.5	2(4)	20487	0	0	55	30	3	2	16.6	47
	7	103.0	2(4)	11611	0	0	53	29	5	3	16.6	45
	-14	102.8	0(0)	9576	0	1	46	28	23	2	15.8	32
	- 7	102.6	0(0)	6440	0	1	37	37	22	3	13.5	32
	0	101.0	0(0)	5232	0	0	61	33	5	1	16.5	48
7S V.P.C.	1	103.6	0(0)	11911	0	0	79	17	1	3	14.8	40
	3	101.6	0(0)	7425	0	0	72	12	12	4	16.0	44
	5	102.8	0(0)	9901	0	0	70	12	13	5	15.8	32
	7	102.6	0(0)	7320	0	0	63	36	1	0	13.5	30
				GROUP III								
	-14	102.6	0(0)	6730	0	2	45	32	15	6	15.0	40
	- 7	101.0	0(0)	11278	0	4	59	23	11	3	16.0	45
	0	102.5	0(0)	11663	0	2	58	19	2	9	16.6	40
1X	1	101.4	2(4)	31162	1	16	69	4	0	9	16.0	46
	3	101.3	2(2)	14519	0	0	77	13	6	4	16.0	47
	5	102.2	2(2)	7243	0	0	44	33	19	4	16.8	44
	7	103.0	2(4)	24783	0	0	80	16	3	1	14.0	43
	-14	103.3	0(0)	7454	0	0	76	15	6	3	16.0	49
	- 7	103.4	0(0)	8497	0	4	74	15	4	2	14.8	40
	0	101.8	0(0)	11905	0	1	53	27	12	7	17.0	45
2X	1	101.2	2(4)	24592	0	22	77	1	0	0	15.0	33
	3	101.7	2(3)	12255	0	0	82	14	1	3	15.0	35
	5	102.2	2(3)	14247	0	0	81	11	5	3	15.2	38
	7	102.9	2(4)	13133	0	0	76	18	4	2	15.6	44

TABLE 1 (cont.)

Dog No.	Day	Temp.	CRP	WBC	Differential leukocyte count						Hb.	PCV
					Juv.	Band	Seg.	Lym.	Eos.	Mon.		
				GROUP III								
	-14	102.5	0(0)	7544	2	2	39	42	13	2	13.8	41
	- 7	102.5	0(0)	10673	0	3	44	43	2	2	18.5	40
	0	102.2	0(0)	10362	0	4	72	18	6	0	14.4	40
3B	1	102.9	1(4)	29377	1	10	90	0	0	0	12.6	38
	3	102.8	1(3)	10754	0	3	61	21	8	7	14.8	40
	5	101.0	0(0)	8646	0	4	58	24	1	4	12.6	40
	7	103.1	0(0)	8719	0	10	40	28	15	6	14.8	40
	-14	102.4	0(0)	8839	0	5	54	32	7	2	17.6	40
	- 7	102.8	0(0)	7452	0	2	47	49	1	1	16.0	43
	0	102.4	1(4)	8248	0	5	56	35	1	3	14.8	36
5B	1	102.4	1(4)	38601	1	10	83	3	3	0	13.4	34
	3	102.4	1(4)	17860	0	2	73	17	3	5	14.8	40
	5	101.2	0(0)	9207	0	6	50	34	3	7	14.2	27
	7	101.7	0(0)	12208	0	7	53	36	1	3	12.6	35
	-14	102.4	0(0)	10353	1	4	68	15	7	5	15.2	38
	- 7	102.2	0(0)	8282	0	4	71	12	5	7	14.4	40
	0	101.9	0(0)	16793	0	1	74	15	7	3	15.2	37
3X C.	1	102.3	2(1)	5895	1	42	32	11	0	14	14.4	31
	3	104.8	2(2)	8466	0	5	66	18	7	5	14.8	29
	5	100.8	2(2)	10233	0	0	61	30	8	1	11.6	22
	7	102.8	2(2)	31845	0	1	83	12	3	1	10.8	40
	-14	102.2	0(0)	4980	0	1	41	47	9	2	16.0	40
	- 7	100.8	0(0)	9605	0	0	52	46	2	0	17.0	44
	0	101.2	0(0)	11383	0	5	66	19	3	6	12.2	34
4X V.P.C.	1	101.0	0(0)	12343	0	4	76	11	4	5	14.4	24
	3	101.1	0(0)	18480	0	0	70	20	7	3	14.0	39
	5	101.5	0(0)	18638	0	0	69	23	7	1	13.4	30
	7	102.6	2(1)	17442	0	0	66	28	3	3	16.4	23
				GROUP IV								
	- 2	102.0	0(0)	10276	0	0	53	40	0	7	*	26
	- 1	102.0	0(0)	11845	0	0	33	65	0	2		
	0	101.2	0(0)	10040	0	0	28	71	1	0		25
2M	2	101.2	0(0)	20098	0	0	69	30	1	0		29
	4	102.0	0(0)	18374	0	0	35	47	3	15		..
	6	102.0	0(0)	27798	0	0	9	91	0	0		31
	8	102.2	0(0)	14413	0	0	16	79	2	3		34
	10	102.0	0(0)	10157	0	0	48	49	0	3		33

* Blood hemoglobin values were not available on Group IV.

TABLE 1 (cont.)

Dog No.	Day	Temp.	CRP	WBC	GROUP IV Differential leukocyte count Juv.	Band	Seg.	Lym.	Eos.	Mon.	Hb.	PCV
	-2	102.0	0(0)	8156	0	2	53	39	3	3		24
	-1	102.6	0(0)	11450	0	0	42	55	2	1		..
	0	101.8	0(0)	9412	0	0	25	67	3	5		25
3M	2	101.4	0(0)	14745	0	0	48	51	0	1		28
	4	102.5	0(0)	13849	0	0	32	62	2	4		..
	6	102.2	0(0)	25027	0	0	21	76	0	3		33
	8	102.4	0(0)	12888	0	0	24	75	0	1		35
	10	102.6	0(0)	9826	0	0	46	50	2	2		28
	-2	101.6	0(0)	9628	0	0	67	28	3	2		38
	-1	101.6	0(0)	7010	0	0	34	62	0	4		
	0	101.4	0(0)	5987	0	0	38	58	1	3		35
4M	2	101.8	0(0)	11783	0	0	44	55	0	1		31
	4	101.4	0(0)	12784	0	0	38	59	2	1		..
	6	102.4	0(0)	7176	0	0	30	68	1	1		33
	8	101.6	0(0)	4998	0	0	38	56	4	2		34
	10	102.6	0(0)	14524	0	0	62	34	0	4		35
	-2	102.2	0(0)	8329	0	0	60	34	0	6		36
	-1	101.8	0(0)	7291	0	0	50	37	10	3		..
	0	101.2	0(0)	7226	0	0	38	54	2	6		31
5M	2	101.6	0(0)	11108	0	0	23	75	0	2		28
	4	102.0	0(0)	10250	0	0	53	44	0	3		..
	6	102.7	0(0)	4718	0	0	58	42	0	0		32
	8	102.2	0(0)	7031	0	2	34	60	0	4		31
	10	102.2	0(0)	10564	0	0	18	80	0	2		30
	-2	102.4	0(0)	16760	0	0	59	34	4	3		35
	-1	102.4	0(0)	19897	0	0	61	32	4	3		..
	0	102.0	0(0)	16424	0	0	52	43	3	2		34
6M	2	102.0	0(0)	15917	0	0	32	58	2	8		28
	4	102.5	0(0)	18374	0	0	37	53	3	7		..
	6	103.8	0(0)	21180	0	0	40	54	2	4		34
	8	103.0	0(0)	22532	0	0	16	79	1	4		36
	10	102.8	0(0)	14853	0	0	29	63	1	6		25
	-2	102.6	0(0)	12104	0	0	66	28	3	3		26
	-1	101.8	0(0)	17796	0	0	50	46	0	4		..
	0	101.2	0(0)	14062	0	0	34	66	0	0		34
7M	2	101.5	0(0)	19998	0	0	57	38	3	2		27
	4	103.0	0(0)	15670	0	0	46	50	1	3		..
	6	103.6	0(0)	14793	0	0	42	54	0	4		33
	8	104.2	0(0)	17052	0	0	42	58	0	0		33
	10	102.6	0(0)	4739	0	0	60	37	0	3		33

TABLE 1 (cont.)

Dog No.	Day	Temp.	CRP	WBC	Differential leukocyte count						Hb.	PCV
					Juv.	Band	Seg.	Lym.	Eos.	Mon.		
GROUP IV												
9M	-2	101.5	0(0)	10510	0	0	60	36	1	3		35
	-1	103.2	0(0)	13402	0	0	53	44	2	1		..
	0	102.2	0(0)	9203	0	0	50	47	1	2		34
	2	101.8	0(0)	15603	0	1	32	64	1	2		31
	4	102.4	0(0)	15026	0	0	41	57	1	1		..
	6	102.0	0(0)	11389	0	0	26	71	0	3		33
	8	101.8	0(0)	15736	0	0	31	65	1	3		34
	10	102.4	0(0)	3827	0	0	63	36	0	1		35
1M C.	-2	101.2	0(0)	10276	0	0	69	24	3	4		26
	-1	101.8	0(0)	15389	0	0	57	36	4	3		..
	0	101.8	0(0)	14990	0	0	60	31	1	8		24
	2	102.0	0(0)	20797	0	0	59	36	0	5		29
	4	102.0	0(0)	15993	0	0	61	29	7	3		..
	6	101.8	0(0)	33970	0	0	40	55	5	0		36
	8	102.4	0(0)	23312	0	0	45	50	5	0		38
	10	102.4	0(0)	11067	0	0	36	62	0	2		39
8M C.	-2	101.2	0(0)	13799	0	0	50	48	0	2		22
	-1	101.2	0(0)	14195	0	0	56	43	0	1		..
	0	101.6	0(0)	14062	0	0	26	74	0	0		25
	2	101.2	0(0)	14057	0	0	49	50	0	1		28
	4	101.4	0(0)	20890	0	3	33	60	0	4		..
	6	101.6	0(0)	22090	0	0	22	75	2	1		33
	8	101.8	0(0)	11835	0	0	51	41	2	6		35
	10	102.0	0(0)	12785	0	0	33	67	0	0		33
10M C.	-2	102.2	0(0)	19054	0	0	73	22	1	4		36
	-1	101.6	0(0)	18152	0	0	71	21	2	6		..
	0	102.0	0(0)	15385	. . . . . Clotted							
	2	101.4	0(0)	14442	0	0	60	29	2	9		30
	4	102.0	0(0)	18919	0	0	69	27	2	2		..
	6	101.8	0(0)	14706	0	0	71	26	2	1		29
	8	102.0	0(0)	9699	0	0	58	38	4	0		31
	10	102.4	0(0)	3827	0	0	11	85	1	3		24
GROUP V												
2M	0	102.4	0(0)	5394	0	0	44	52	22	0	14.0	30
	2	102.8	0(0)	5336	0	0	50	45	5	0	12.2	30
	4	104.0	0(0)	6228	0	10	82	8	0	0	11.2	33
	6	102.0	0(0)	3922	0	1	50	48	1	0	13.7	30
	8	102.0	4(1)	6500	0	0	94	51	0	1	16.0	30
	10	104.0	2(1)	74677	0	51	88	4	0	2	14.8	31
	12	102.2	1(1)	8070	0	1	74	22	0	3	9.8	27

TABLE 1 (cont.)

Dog No.	Day	Temp.	CRP	WBC	Differential leukocyte count						Hb.	PCV
					Juv.	Band	Seg.	Lym.	Eos.	Mon.		
				GROUP V								
	0	103.0	0(0)	4464	0	0	44	46	10	0	13.6	29
	2	102.0	0(0)	9181	0	0	66	22	0	12	11.0	31
	4	102.6	0(0)	9438	0	0	46	36	16	2	11.2	35
3M	6	102.0	0(0)	6297	0	1	37	52	4	6	14.6	35
	8	103.0	0(0)	6800	0	0	23	57	20	0	12.2	33
	10	102.0	2(1)	9024	0	0	51	36	13	0	13.4	31
	12	101.8	0(0)	9653	0	0	44	32	24	0	11.6	33
	0	102.8	0(0)	3309	0	2	50	34	12	2	11.0	35
	2	102.0	0(0)	4856	0	4	70	22	2	2	14.0	35
	4	102.6	0(0)	8485	0	1	70	25	3	1	12.6	39
4M	6	101.6	1(2)	6150	0	0	53	44	2	1	12.6	34
	8	102.0	0(0)	6800	0	0	70	20	10	0	12.2	40
	10	101.6	2(1)	57277	0	5	54	30	10	1	14.0	35
	12	102.0	0(0)	6422	0	1	54	36	6	3	12.0	35
	0	101.4	0(0)	4392	0	0	52	46	2	0	11.4	37
	2	102.4	0(0)	5749	0	0	25	72	3	0	12.2	35
	4	102.0	0(0)	8299	0	0	50	42	3	5	12.2	37
5M	6	102.8	0(0)	7450	0	4	60	34	0	2	13.0	37
	8	102.0	0(0)	7850	0	0	63	34	1	2	14.0	37
	10	101.0	2(1)	5072	0	2	55	35	1	7	14.0	35
	12	101.4	0(0)	7668	0	0	58	40	0	2	12.0	33
	0	103.0	0(0)	8131	0	0	58	30	0	12	12.2	33
	2	101.8	0(0)	10548	0	0	58	30	10	2	13.0	35
	4	101.4	0(0)	15064	0	0	56	30	12	2	12.2	37
6M	6	102.0	0(0)	11100	0	0	58	32	10	0	18.2	40
	8	101.2	0(0)	6800	0	1	61	35	1	2	14.0	39
	10	101.0	2(1)	10810	0	0	50	36	7	7	12.2	32
	12	102.0	0(0)	10145	0	0	53	34	6	6	12.0	32
	0	102.4	0(0)	6322	0	0	42	46	6	6	12.2	37
	2	101.8	0(0)	5154	0	0	48	50	2	0	16.0	40
	4	101.4	0(0)	6837	0	2	60	26	10	2	13.0	36
9M	6	101.2	1(3)	8050	0	2	68	20	8	2	14.0	38
	8	101.0	2(1)	8000	0	0	46	50	2	2	13.8	40
	10	101.0	2(2)	8012	0	1	50	36	7	6	13.6	38
	12	102.0	0(0)	8489	0	0	48	46	6	0	13.0	36
	0	102.8	0(0)	4332	0	2	62	28	4	2	12.6	35
	2	102.6	0(0)	9025	0	0	77	22	1	0	12.6	35
	4	104.8	0(0)	10521	0	6	90	4	0	0	16.4	37
1M C.	6	103.6	1(1)	6466	0	0	80	16	0	4	15.6	34
	8	105.8	8(2)	20200	0	0	82	18	0	0	13.6	36
	10	Died										
	12											

TABLE 1 (cont.)

Dog No.	Day	Temp.	CRP	WBC	Differential leukocyte count						Hb.	PCV
					Juv.	Band	Seg.	Lym.	Eos.	Mon.		
GROUP V												
8M C.	0	102.8	0(0)	8464	0	1	24	67	5	3	11.2	30
	2	101.8	0(0)	12698	0	0	41	52	3	0	12.0	32
	4	103.4	0(0)	4725	0	0	84	12	0	4	12.0	35
	6	102.8	1(1)	8650	0	1	79	16	2	2	12.0	26
	8	Died										
	10											
	12											
10M C.	0	102.0	0(0)	7981	0	3	61	25	3	8	15.6	39
	2	101.4	0(0)	9995	0	2	68	28	0	2	13.0	35
	4	104.8	0(0)	7677	0	0	84	16	0	0	13.6	32
	6	104.6	1(2)	4800	0	8	72	16	0	4	13.3	30
	8	106.8	2(1)	8000	0	0	80	12	5	3	16.0	30
	10	Euthanatized										
	12											
GROUP VI												
2MM	-14	101.8	0(0)	4921	0	0	72	25	2	1	10.8	34
	- 7	104.0	0(0)	5054	0	0	78	20	1	1	12.6	29
	0	102.2	0(0)	8923	0	1	57	32	5	5	11.0	32
	1	102.6	2(1)	7298	0	16	46	23	0	15	11.2	38
	3	104.8	1(1)	7359	0	27	35	23	0	15	10.8	33
	5	102.0	2(3)	8120	0	15	45	30	0	10	12.0	34
	7	102.0	1(3)	11800	0	3	63	25	5	4	11.0	33
4MM	-14	101.9	0(0)	6500	0	0	64	27	9	0	11.6	36
	- 7	101.8	0(0)	6291	0	0	70	21	7	2	11.0	30
	0	102.4	0(0)	7501	0	21	42	42	11	3	13.2	44
	1	102.2	8(1)	16211	0	15	67	16	1	1	16.0	49
	3	103.2	8(3)	8920	0	5	71	11	1	12	12.0	38
	5	103.4	8(1)	9151	0	5	78	10	6	1	11.0	32
	7	101.6	2(3)	14600	0	8	63	21	7	1	14.4	38
6MM	-14	102.3	0(0)	10364	0	0	58	34	5	3	12.6	35
	- 7	102.2	0(0)	11509	0	0	61	29	10	0	14.0	38
	0	103.0	0(0)	9963	0	2	33	39	18	8	14.8	42
	1	101.6	8(1)	36072	0	19	72	17	2	0	11.6	41
	3	102.0	4(1)	8513	0	2	50	43	3	2	14.0	39
	5	103.0	4(1)	8732	0	4	52	40	3	1	13.6	40
	7	102.0	2(2)	18500	0	2	54	39	1	4	13.6	40

TABLE 1 (concl.)

GROUP VI												
Dog No.	Day	Temp.	CRP	WBC	Differential leukocyte count						Hb.	PCV
					Juv.	Band	Seg.	Lym.	Eos.	Mon.		
	-14	102.0	0(0)	6624	0	0	52	34	11	3	14.0	33
	- 7	101.8	0(0)	5511	0	0	43	51	6	0	14.4	38
	0	102.4	0(0)	9178	0	0	64	30	2	4	15.6	44
9MM	1	102.5	4(3)	13845	0	11	68	21	0	0	15.2	48
	3	104.0	8(2)	7789	0	8	68	18	0	6	15.2	41
	5	102.4	4(1)	9163	0	9	64	20	3	4	14.0	40
	7	101.6	4(2)	14400	0	8	66	18	8	0	14.8	44
	-14	103.0	0(0)	7565	0	0	44	26	27	3	11.0	35
	- 7	102.0	0(0)	8541	0	0	51	20	25	4	12.0	24
	0	103.0	0(0)	9375	0	0	48	36	11	5	13.6	41
3MM C.	1	102.6	2(1)	11912	0	8	38	34	18	2	12.0	39
	3	102.0	4(2)	9904	0	1	59	28	11	1	12.0	35
	5	102.0	2(3)	11600	0	6	45	35	10	4	12.4	35
	7	102.0	1(2)	15700	0	4	62	19	8	7	12.0	35
	-14	102.2	0(0)	5956	0	1	63	32	1	3	12.2	35
	- 7	101.4	0(0)	6035	0	0	71	27	1	1	13.0	36
	0	103.0	0(0)	8870	0	0	67	30	0	3	14.8	43
5MM C.	1	102.8	4(1)	9154	0	4	60	36	0	0	14.8	42
	3	103.0	2(2)	8777	0	4	57	30	0	9	14.0	38
	5	102.0	2(1)	9028	0	5	60	30	3	2	14.6	43
	7	102.4	2(3)	9400	0	5	55	38	0	2	13.4	37
	-14	103.0	0(0)	13829	0	4	61	22	14	1	15.8	40
	- 7	102.6	0(0)	6163	0	2	51	20	25	2	13.0	32
	0	100.6	0(0)	8282	0	0	45	37	5	3	14.2	32
5X V.P.C.	1	102.0	0(0)	14943	0	4	56	24	12	4	12.6	40
	3	101.5	0(0)	7534	0	0	67	29	2	2	14.8	44
	5	102.0	0(0)	14810	4	4	74	8	0	10	14.0	40
	7	101.4	0(0)	8285	0	8	69	15	4	4	11.2	33

TABLE 2. A Summary of the C-Reactive Protein Titrations and Serum Fractions of 283 Canine Serum Samples in Six Groups of Experimental Animals.

Dog No.	Day	CRP Titer	% Albumin	% Globulins			
				Alpha-1	Alpha-2	Beta	Gamma
GROUP I							
	-14	0	32.3	5.9	24.2	10.8	26.9
	- 7	0	32.1	4.3	21.4	16.3	25.9
	0	1	34.0	9.2	20.0	12.5	24.4
1B	1	2	30.5	9.3	22.1	14.6	23.5
	3	0	34.0	6.4	20.5	12.8	26.3
	5	0	31.0	7.7	21.0	13.4	27.0
	7	0	39.5	8.0	17.6	15.2	19.6
	-14	0	39.6	11.9	16.2	5.9	26.3
	- 7	0	36.5	7.8	10.2	8.1	37.4
	0	0	46.0	6.4	17.6	5.9	24.2
4B	1	2	29.2	11.7	19.7	14.2	25.2
	3	0	39.4	7.8	15.5	14.5	22.8
	5	0	35.4	8.1	18.3	11.5	26.6
	7	0	36.9	8.0	17.9	9.9	27.3
	-14	0	35.8	17.0	9.7	12.9	24.6
	- 7	0	35.2	16.4	12.1	7.7	28.6
	0	0	41.1	5.7	16.4	12.6	24.3
2B	1	0	34.0	6.7	10.7	9.4	39.1
Control	3	0	35.2	16.0	12.5	9.7	26.6
	5	0	36.8	16.0	8.8	13.8	24.6
	7	0	34.2	6.9	10.5	9.0	39.3
	-14	0	25.2	7.2	14.1	4.4	49.1
	- 7	0	38.0	3.8	19.7	14.9	23.6
	0	0	31.4	8.8	11.5	6.1	42.2
6B	1	0	32.2	9.0	14.2	14.0	30.7
V.P. Control	3	0	41.9	6.7	24.9	6.4	20.0
	5	0	32.4	8.8	13.9	14.3	30.7
	7	0	35.7	4.7	12.5	4.7	42.3
GROUP II							
	-14	0	49.2	14.7	8.8	8.1	19.1
	- 7	0	41.9	12.7	16.6	8.7	20.1
	0	0	52.4	6.7	16.0	6.7	18.1
1S	1	0	45.8	10.4	20.8	7.8	15.2
	3	0	43.0	12.3	23.2	7.5	14.0
	5	2	48.6	5.9	19.6	8.4	17.6
	7	2	48.9	7.2	17.4	8.5	18.0

TABLE 2 (cont.)

Dog No.	Day	CRP Titer	% Albumin	% Globulins Alpha-1	Alpha-2	Beta	Gamma
				GROUP II			
	-14	0	37.3	8.9	27.5	5.9	20.4
	- 7	0	39.7	12.4	20.1	7.7	20.0
	0	0	42.9	9.1	19.4	10.9	17.6
3S	1	0	31.9	18.2	25.9	8.9	15.0
	3	0	42.0	6.4	21.5	8.3	21.8
	5	0	48.6	5.6	15.3	8.8	21.7
	7	2	40.4	11.6	16.7	10.2	21.1
	-14	0	43.0	7.1	20.0	7.8	22.0
	- 7	0	34.0	10.2	23.5	7.2	25.0
5S	0	0	35.5	8.0	23.3	8.0	25.3
Control	1	0	35.5	8.4	23.5	6.1	26.5
	3	0	30.1	9.7	27.5	11.9	20.8
	5	2	43.9	5.0	14.3	11.5	25.2
	7	2	42.4	4.9	18.4	13.3	21.0
	-14	0	41.0	14.5	15.1	8.3	21.0
	- 7	0	45.5	8.7	15.1	7.9	22.7
7S	0	0	45.5	9.0	18.7	6.7	19.9
V.P. Control	1	0	34.5	12.7	20.3	10.5	21.9
	3	0	33.3	11.6	24.4	8.1	21.6
	5	0	36.6	12.8	18.2	8.2	24.1
	7	0	38.2	12.0	21.2	9.0	19.5
				GROUP III			
	-14	0	40.8	4.7	14.9	14.9	24.8
	- 7	0	29.5	6.1	17.7	23.7	22.9
	0	0	32.6	9.9	24.1	9.6	23.8
1X	1	2	35.8	6.3	25.1	11.5	21.4
	3	2	31.3	6.2	26.0	19.2	17.2
	5	2	34.1	6.6	22.9	18.2	18.8
	7	2	32.8	5.9	23.6	16.3	21.4
	-14	0	43.5	5.4	17.5	6.7	26.9
	- 7	0	32.1	6.1	25.5	8.8	27.4
	0	0	27.1	9.0	23.2	15.8	24.9
2X	1	2	35.3	5.3	24.8	12.8	21.8
	3	2	29.4	5.9	19.4	20.0	25.3
	5	2	39.7	2.7	24.4	11.7	21.5
	7	2	29.7	4.3	19.1	9.4	37.5

TABLE 2 (cont.)

Dog No.	Day	CRP Titer	% Albumin	% Globulins Alpha-1	Alpha-2	Beta	Gamma
			GROUP III				
	-14	0	28.5	8.2	20.6	17.7	25.0
	- 7	0	40.5	10.8	17.1	11.4	20.1
	0	0	36.6	4.4	18.7	13.7	26.6
3B	1	1	37.1	5.7	20.3	10.9	26.1
	3	1	36.0	4.2	21.6	12.2	25.9
	5	0	26.5	9.0	26.5	13.3	24.7
	7	0	24.1	12.3	29.4	10.7	23.5
	-14	0	35.9	11.2	16.3	9.5	27.1
	- 7	0	39.5	4.1	22.6	11.6	22.1
	0	1	36.0	3.3	18.9	15.6	26.3
5B	1	1	34.9	5.9	18.3	13.0	27.9
	3	1	29.7	5.2	21.4	12.4	31.4
	5	0	27.2	7.6	28.0	11.2	26.0
	7	0	20.5	11.5	26.4	13.6	28.0
	-14	0	34.2	7.5	18.5	11.6	28.1
	- 7	0	27.5	6.0	19.1	14.1	33.3
	0	0	28.1	8.3	17.2	19.2	27.2
3X Control	1	2	31.0	4.5	12.2	17.4	34.8
	3	2	24.8	6.6	15.3	24.1	29.2
	5	2	20.8	5.4	14.8	25.5	33.6
	7	2	19.8	4.7	15.4	28.4	31.7
	-14	0	42.7	7.7	9.1	9.9	30.5
	- 7	0	36.6	6.9	17.9	9.6	28.9
	0	0	25.3	4.3	11.2	19.3	39.9
4X V.P. Control	1	0	30.2	5.9	16.2	18.4	29.2
	3	0	23.1	10.0	28.9	15.1	22.8
	5	0	32.4	7.7	13.3	15.5	31.0
	7	2	35.7	7.6	18.2	11.1	27.3
			GROUP IV				
	-2	0	52.5	9.4	10.9	11.8	15.4
	-1	0	54.3	7.7	12.8	7.7	17.6
	0	0	52.4	8.6	16.3	11.2	11.4
2M	2	0	51.7	9.8	14.9	6.7	17.0
	4	0	52.1	10.6	12.3	7.9	17.2
	6	0	53.7	11.9	9.7	10.6	14.1
	8	0	53.9	8.7	12.6	7.1	17.7

TABLE 2 (cont.)

GROUP IV							
Dog No.	Day	CRP Titer	% Albumin	% Globulins Alpha-1	Alpha-2	Beta	Gamma
	-2	0	55.0	10.8	10.4	9.5	14.2
	-1	0	64.2	8.6	11.8	7.1	18.2
	0	0	52.9	10.4	12.0	8.2	16.4
3M	2	0	52.9	10.5	10.2	9.1	17.2
	4	0	51.3	12.6	12.4	9.5	14.2
	6	0	54.5	11.0	12.5	6.3	15.7
	8	0	47.5	12.2	13.1	10.0	17.2
	-2	0	55.8	5.6	13.2	10.1	14.3
	-1	0	58.7	8.0	12.0	7.2	14.1
	0	0	50.4	11.0	16.1	7.1	15.4
4M	2	0	54.1	10.1	11.4	7.3	17.0
	4	0	50.8	8.5	14.5	7.8	18.3
	6	0	49.6	8.1	16.1	10.2	16.0
	8	0	44.0	9.5	16.6	9.9	19.9
	-2	0	58.6	10.3	9.8	9.3	11.9
	-1	0	58.0	10.0	12.3	7.3	12.3
	0	0	54.1	10.9	10.9	9.3	14.7
5M	2	0	51.1	7.7	14.4	16.1	10.7
	4	0	57.5	9.0	12.2	8.5	12.8
	6	0	47.2	7.9	16.8	6.5	21.5
	8	0	51.0	7.0	16.3	7.0	18.7
	-2	0	61.0	8.1	10.5	5.7	14.7
	-1	0	62.0	5.6	8.8	8.8	14.7
	0	0	60.3	6.4	10.7	8.6	14.0
6M	2	0	60.0	6.4	11.9	5.9	15.8
	4	0	52.9	7.6	10.9	8.7	19.8
	6	0	59.0	6.2	10.0	8.0	16.8
	8	0	55.4	7.4	9.2	8.3	19.6
	-2	0	58.8	5.1	9.0	6.8	20.2
	-1	0	57.9	6.8	9.3	8.1	17.8
	0	0	56.4	7.9	11.4	6.4	17.9
7M	2	0	56.0	9.7	10.8	6.7	16.8
	4	0	56.3	13.8	5.9	5.4	18.5
	6	0	54.2	9.9	15.6	3.6	16.6
	8	0	50.8	6.9	15.0	8.6	18.7
	-2	0	62.4	7.6	8.1	9.7	12.2
	-1	0	57.2	6.6	12.1	12.1	12.1
	0	0	50.7	12.6	13.8	6.9	16.0
9M	2	0	50.8	6.2	13.6	13.6	15.8
	4	0	58.5	6.8	10.4	9.4	14.8
	6	0	54.0	8.0	14.6	9.6	14.4
	8	0	57.7	8.4	9.8	9.8	14.3

TABLE 2 (cont.)

Dog No.	Day	CRP Titer	% Albumin	% Globulins Alpha-1	Alpha-2	Beta	Gamma
			GROUP IV				
	-2	0	56.2	8.4	13.2	6.0	16.3
	-1	0	47.9	13.7	9.3	7.5	21.6
1M	0	0	48.8	13.2	11.9	7.3	18.7
Control	2	0	49.9	14.2	11.0	7.7	17.2
	4	0	52.7	8.4	10.4	9.4	18.9
	6	0	50.1	12.2	8.2	7.9	21.5
	8	0	49.3	10.8	11.9	7.2	20.8
	-2	0	61.3	8.5	8.6	5.3	16.3
	-1	0	55.8	11.2	6.8	10.6	15.6
8M	0	0	58.0	9.8	8.0	10.9	13.3
Control	2	0	45.1	12.5	11.6	14.3	16.4
	4	0	56.3	10.0	10.8	8.5	14.4
	6	0	55.4	10.7	8.9	7.7	17.2
	8	0	50.9	10.8	9.0	7.3	21.9
	-2	0	63.1	5.8	9.5	6.9	14.7
	-1	0	53.9	7.8	14.8	9.4	14.1
10M	0	0	54.4	8.6	10.8	18.3	8.0
Control	2	0	50.9	7.0	16.8	7.9	17.4
	4	0	54.5	5.7	14.6	9.0	16.2
	6	0	51.0	8.9	12.5	7.1	20.5
	8	0	53.9	7.7	12.2	5.6	20.6
			GROUP V				
	0	0	51.4	9.4	13.5	7.3	18.4
	2	0	44.8	16.1	20.3	5.1	13.8
	4	0	44.4	10.7	25.0	5.6	14.3
2M	6	0	45.3	8.2	21.4	7.5	17.6
	8	4	50.2	5.2	23.4	7.1	14.1
	10	2	43.4	5.9	29.4	5.9	15.5
	12	1	46.0	5.6	24.5	8.2	15.7
	0	0	57.4	6.5	16.5	7.0	12.6
	2	0	52.5	8.3	19.3	5.2	14.6
	4	0	54.5	10.4	15.7	3.7	15.7
3M	6	0	54.3	5.4	15.4	6.3	18.6
	8	0	52.6	7.8	19.8	6.0	13.8
	10	2	54.2	7.7	19.0	3.9	15.1
	12	0	54.3	8.9	15.6	4.1	17.1

TABLE 2 (cont.)

Dog No.	Day	CRP Titer	% Albumin	% Globulins Alpha-1	Alpha-2	Beta	Gamma
			GROUP V				
	0	0	49.2	4.8	17.7	9.7	18.5
	2	0	54.1	7.4	14.8	11.9	11.9
	4	0	34.5	22.1	23.4	9.6	10.3
4M	6	1	49.3	9.8	18.8	9.0	13.1
	8	0	47.5	19.8	12.5	6.3	13.9
	10	2	48.1	14.1	11.8	12.5	13.5
	12	0	45.3	10.0	23.4	8.5	12.9
	0	0	54.1	6.4	16.2	7.1	16.2
	2	0	52.9	6.5	18.1	7.2	15.2
	4	0	38.4	14.0	23.1	6.3	18.2
5M	6	0	53.9	6.2	18.8	8.6	12.5
	8	0	45.0	10.5	24.0	7.5	13.0
	10	2	50.1	6.4	17.6	8.8	17.0
	12	0	44.5	12.5	19.5	8.2	15.4
	0	0	55.3	5.9	15.3	8.1	15.4
	2	0	40.5	9.2	20.1	11.6	18.6
	4	0	49.4	8.7	16.2	9.3	16.4
6M	6	0	48.6	7.4	13.6	11.1	19.2
	8	0	44.1	5.7	15.8	10.6	23.8
	10	2	48.3	3.9	16.2	6.9	24.7
	12	0	39.8	11.3	18.5	9.7	20.7
	0	0	55.1	6.5	15.4	8.9	14.1
	2	0	42.1	13.7	24.4	5.5	14.3
	4	0	47.8	8.3	22.3	7.6	13.9
9M	6	1	55.7	8.2	15.2	6.8	14.0
	8	2	51.6	5.4	22.2	8.4	12.4
	10	2	49.5	7.3	16.3	12.5	14.3
	12	0	44.9	12.1	19.5	7.8	15.7
	0	0	52.3	8.4	11.0	9.7	18.7
	2	0	55.6	7.9	19.8	6.0	10.7
1M	4	0	47.9	7.4	21.4	11.4	12.0
Control	6	1	38.4	11.0	25.7	9.8	15.2
	8	8	47.6	5.9	23.5	9.5	13.6
	10	Died					
	12						
	0	0	54.8	6.6	12.6	7.9	18.0
	2	0	50.0	7.9	19.8	6.3	16.0
8M	4	0	47.3	7.8	20.0	8.9	16.0
Control	6	1	43.3	7.0	25.6	8.5	15.5
	8	Died					
	10						
	12						

TABLE 2 (cont.)

Dog No.	Day	CRP Titer	% Albumin	% Globulins Alpha-1	Alpha-2	Beta	Gamma
			GROUP V				
	0	0	44.0	9.0	14.2	9.7	23.1
	2	0	35.4	19.2	23.8	6.1	15.6
	4	0	45.4	6.8	21.6	9.1	17.1
10M Control	6	1	45.4	5.0	27.6	5.0	16.9
	8	2	39.8	6.0	30.0	6.6	17.5
	10	Euthanatized					
	12						
			GROUP VI				
	-14	0	41.0	8.3	18.9	10.6	21.2
	- 7	0	35.2	13.5	17.4	9.7	24.1
	0	0	41.2	8.5	19.3	10.0	20.9
2MM	1	2	34.4	8.2	16.4	11.8	29.3
	3	1	33.7	7.7	24.2	10.0	24.3
	5	2	31.9	8.7	22.4	13.3	23.7
	7	1	39.3	6.2	20.0	14.3	20.2
	-14	0	49.0	9.0	15.8	9.0	17.1
	- 7	0	46.4	7.8	17.3	13.0	15.4
	0	0	38.3	13.3	19.3	10.1	19.1
4MM	1	8	44.4	7.7	14.9	13.9	19.0
	3	8	31.2	8.7	22.4	19.1	18.6
	5	8	30.2	5.2	24.0	19.3	21.2
	7	2	34.4	5.9	21.2	16.1	22.3
	-14	0	42.3	7.3	18.2	12.2	20.0
	- 7	0	48.8	5.6	16.2	13.1	16.3
	0	0	44.2	8.1	13.9	13.2	20.5
6MM	1	8	41.3	9.0	16.4	10.5	22.9
	3	4	40.4	7.2	15.1	13.1	24.1
	5	4	40.1	5.9	14.0	14.0	25.9
	7	2	44.8	6.5	13.1	13.1	22.5
	-14	0	47.3	6.2	15.3	11.5	19.7
	- 7	0	45.4	6.4	16.1	12.9	19.1
	0	0	42.9	9.5	21.7	8.1	17.9
9MM	1	4	36.8	13.6	19.6	12.8	17.2
	3	8	34.3	10.0	22.7	14.0	18.9
	5	4	37.0	7.9	23.6	14.7	16.7
	7	4	39.6	6.7	20.7	14.8	18.2

TABLE 2 (concl.)

GROUP VI							
Dog No.	Day	CRP Titer	% Albumin	% Globulins			
				Alpha-1	Alpha-2	Beta	Gamma
3MM Control	-14	0	43.5	9.9	19.9	8.7	18.0
	- 7	0	49.9	8.7	16.5	9.7	15.1
	0	0	45.0	7.2	16.3	9.8	21.7
	1	2	54.7	7.7	12.6	8.4	16.5
	3	4	54.5	9.1	10.7	8.3	17.5
	5	2	48.5	4.6	14.5	8.4	23.9
	7	1	49.8	5.4	11.5	10.7	22.6
5MM Control	-14	0	47.5	7.0	20.3	9.6	15.5
	- 7	0	51.3	8.6	15.7	11.0	13.3
	0	0	44.9	11.0	18.8	8.1	17.1
	1	4	50.9	9.4	13.4	8.0	18.3
	3	2	50.3	9.4	12.2	10.6	17.5
	5	2	51.3	5.7	14.3	9.1	19.6
	7	2	59.1	8.3	12.1	9.8	10.7
5X V.P. Control	-14	0	33.5	11.4	20.5	10.0	24.5
	- 7	0	33.5	4.8	17.5	14.5	29.7
	0	0	27.3	9.1	13.6	14.3	35.7
	1	0	28.5	21.3	10.0	14.3	25.8
	3	0	42.1	6.2	14.6	13.5	23.5
	5	0	26.1	5.7	20.5	21.5	26.1
	7	0	31.5	6.4	20.6	12.8	28.6

TABLE 3. A Summary of the C-Reactive Protein Stability in Fresh, Refrigerated, and Frozen Canine Serum Samples in Six Groups of Experimental Animals.

Dog No.	Day	CRP reaction and titer* Fresh 1 2 4 8	Refrigerated 1 2 4 8	Frozen 1 2 4 8
	GROUP I			
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
	0	1 0 0 0	0 0 0 0	0 0 0 0
1B	1	1 1 0 0	1 0 0 0	0 0 0 0
	3	0 0 0 0	0 0 0 0	0 0 0 0
	5	0 0 0 0	0 0 0 0	0 0 0 0
	7	0 0 0 0	0 0 0 0	0 0 0 0
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
4B	1	1 1 0 0	1 1 0 0	1 1 0 0
	3	0 0 0 0	0 0 0 0	0 0 0 0
	5	0 0 0 0	0 0 0 0	0 0 0 0
	7	0 0 0 0	0 0 0 0	0 0 0 0
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
2B Control	1	0 0 0 0	0 0 0 0	0 0 0 0
	3	0 0 0 0	0 0 0 0	0 0 0 0
	5	0 0 0 0	0 0 0 0	0 0 0 0
	7	0 0 0 0	0 0 0 0	0 0 0 0
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
6B V.P. Control	1	0 0 0 0	0 0 0 0	0 0 0 0
	3	0 0 0 0	0 0 0 0	0 0 0 0
	5	0 0 0 0	0 0 0 0	0 0 0 0
	7	0 0 0 0	0 0 0 0	0 0 0 0
	GROUP II			
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
1S	1	0 0 0 0	0 0 0 0	0 0 0 0
	3	0 0 0 0	0 0 0 0	0 0 0 0
	5	3 2 0 0	3 2 0 0	3 1 0 0
	7	2 1 0 0	2 1 0 0	2 0 0 0

* The columns 1, 2, 4, 8 represent the C-reactive protein titer, and the number in the column represents the strength of the agglutination reaction.

TABLE 3 (cont.)

Dog No.	Day	CRP reaction and titer: Fresh 1 2 4 8	Refrigerated 1 2 4 8	Frozen 1 2 4 8
		GROUP II		
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
3S	1	0 0 0 0	0 0 0 0	0 0 0 0
	3	0 0 0 0	0 0 0 0	0 0 0 0
	5	0 0 0 0	0 0 0 0	0 0 0 0
	7	4 4 0 0	4 4 0 0	4 4 0 0
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
5S	1	0 0 0 0	0 0 0 0	0 0 0 0
Control	3	0 0 0 0	0 0 0 0	0 0 0 0
	5	4 4 0 0	4 4 0 0	4 4 0 0
	7	4 4 0 0	4 4 0 0	4 4 0 0
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
7S	1	0 0 0 0	0 0 0 0	0 0 0 0
V.P. Control	3	0 0 0 0	0 0 0 0	0 0 0 0
	5	0 0 0 0	0 0 0 0	0 0 0 0
	7	0 0 0 0	0 0 0 0	0 0 0 0
		GROUP III		
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
1X	1	4 4 0 0	4 4 0 0	4 4 0 0
	3	4 2 0 0	4 2 0 0	3 1 0 0
	5	4 2 0 0	4 2 0 0	4 2 0 0
	7	4 4 0 0	4 4 0 0	4 4 0 0
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
2X	1	4 4 0 0	4 4 0 0	4 4 0 0
	3	4 3 0 0	4 3 0 0	4 3 0 0
	5	4 3 0 0	4 3 0 0	4 3 0 0
	7	4 4 0 0	4 4 0 0	4 4 0 0

TABLE 3 (cont.)

GROUP III

Dog No.	Day	CRP reaction and titer: Fresh 1 2 4 8	Refrigerated 1 2 4 8	Frozen 1 2 4 8
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
3B	1	4 0 0 0	4 0 0 0	3 0 0 0
	3	3 0 0 0	3 0 0 0	3 0 0 0
	5	0 0 0 0	0 0 0 0	0 0 0 0
	7	0 0 0 0	0 0 0 0	0 0 0 0
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
	0	4 0 0 0	0 0 0 0	0 0 0 0
5B	1	4 0 0 0	4 0 0 0	4 0 0 0
	3	4 0 0 0	4 0 0 0	4 0 0 0
	5	0 0 0 0	0 0 0 0	0 0 0 0
	7	0 0 0 0	0 0 0 0	0 0 0 0
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
3X	0	0 0 0 0	0 0 0 0	0 0 0 0
Control	1	1 1 0 0	1 1 0 0	1 0 0 0
	3	2 2 0 0	2 2 0 0	2 2 0 0
	5	2 2 0 0	2 2 0 0	2 2 0 0
	7	2 2 0 0	2 2 0 0	2 2 0 0
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
4X	0	0 0 0 0	0 0 0 0	0 0 0 0
V.P. Control	1	0 0 0 0	0 0 0 0	0 0 0 0
	3	0 0 0 0	0 0 0 0	0 0 0 0
	5	0 0 0 0	0 0 0 0	0 0 0 0
	7	1 1 0 0	1 1 0 0	1 0 0 0

GROUP IV

All serum samples had been frozen for 4 mos. and were all negative.

GROUP V

	0	0 0 0 0	0 0 0 0	0 0 0 0
	2	0 0 0 0	0 0 0 0	0 0 0 0
	4	0 0 0 0	0 0 0 0	0 0 0 0
2M	6	0 0 0 0	0 0 0 0	0 0 0 0
	8	3 2 1 0	3 2 1 0	3 2 0 0
	10	4 1 0 0	4 1 0 0	4 1 0 0
	12	1 0 0 0	0 0 0 0	0 0 0 0

TABLE 3 (cont.)

GROUP V				
		CRP reaction and titer		
Dog No.	Day	Fresh	Refrigerated	Frozen
		1 2 4 8	1 2 4 8	1 2 4 8
	0	0 0 0 0	0 0 0 0	0 0 0 0
	2	0 0 0 0	0 0 0 0	0 0 0 0
	4	0 0 0 0	0 0 0 0	0 0 0 0
3M	6	0 0 0 0	0 0 0 0	0 0 0 0
	8	0 0 0 0	0 0 0 0	0 0 0 0
	10	2 1 0 0	2 1 0 0	2 0 0 0
	12	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
	2	0 0 0 0	0 0 0 0	0 0 0 0
	4	0 0 0 0	0 0 0 0	0 0 0 0
4M	6	2 0 0 0	2 0 0 0	2 0 0 0
	8	0 0 0 0	0 0 0 0	0 0 0 0
	10	3 1 0 0	3 1 0 0	3 0 0 0
	12	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
	2	0 0 0 0	0 0 0 0	0 0 0 0
	4	0 0 0 0	0 0 0 0	0 0 0 0
5M	6	0 0 0 0	0 0 0 0	0 0 0 0
	8	0 0 0 0	0 0 0 0	0 0 0 0
	10	3 1 0 0	3 1 0 0	3 0 0 0
	12	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
	2	0 0 0 0	0 0 0 0	0 0 0 0
	4	0 0 0 0	0 0 0 0	0 0 0 0
6M	6	0 0 0 0	0 0 0 0	0 0 0 0
	8	0 0 0 0	0 0 0 0	0 0 0 0
	10	1 1 0 0	1 1 0 0	0 0 0 0
	12	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
	2	0 0 0 0	0 0 0 0	0 0 0 0
	4	0 0 0 0	0 0 0 0	0 0 0 0
9M	6	3 0 0 0	3 0 0 0	3 0 0 0
	8	1 1 0 0	1 1 0 0	1 0 0 0
	10	4 2 0 0	4 2 0 0	4 2 0 0
	12	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
	2	0 0 0 0	0 0 0 0	0 0 0 0
	4	0 0 0 0	0 0 0 0	0 0 0 0
1M Control	6	1 0 0 0	1 0 0 0	1 0 0 0
	8	4 4 3 2	4 4 3 2	4 3 2 1
	10	Died		
	12			

TABLE 3 (cont.)

Dog No.	Day	CRP reaction and titer: Fresh 1 2 4 8	Refrigerated 1 2 4 8	Frozen 1 2 4 8
		GROUP V		
	0	0 0 0 0	0 0 0 0	0 0 0 0
	2	0 0 0 0	0 0 0 0	0 0 0 0
	4	0 0 0 0	0 0 0 0	0 0 0 0
8M	6	1 0 0 0	1 0 0 0	1 0 0 0
Control	8	Died		
	10			
	12			
	0	0 0 0 0	0 0 0 0	0 0 0 0
	2	0 0 0 0	0 0 0 0	0 0 0 0
	4	0 0 0 0	0 0 0 0	0 0 0 0
10M	6	2 0 0 0	2 0 0 0	2 0 0 0
Control	8	1 1 0 0	1 1 0 0	1 1 0 0
	10	Euthanatized		
	12			
		GROUP VI		
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
2MM	1	3 1 0 0	3 1 0 0	3 0 0 0
	3	1 0 0 0	1 0 0 0	0 0 0 0
	5	3 3 0 0	3 3 0 0	3 3 0 0
	7	3 0 0 0	3 0 0 0	3 0 0 0
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
4MM	1	4 4 3 1	4 4 3 1	4 4 3 0
	3	4 4 4 3	4 4 4 3	4 4 4 3
	5	4 4 3 1	4 4 3 1	4 4 3 0
	7	4 3 0 0	4 3 0 0	4 3 0 0
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
6MM	1	4 4 3 1	4 4 3 1	4 4 3 0
	3	3 2 1 0	3 2 1 0	3 2 1 0
	5	3 2 1 0	3 2 1 0	3 2 1 0
	7	3 2 0 0	3 2 0 0	3 2 0 0

TABLE 3 (concl.)

GROUP VI				
		CRP reaction and titer		
Dog No.	Day	Fresh 1 2 4 8	Refrigerated 1 2 4 8	Frozen 1 2 4 8
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
	0	0 0 0 0	0 0 0 0	0 0 0 0
9MM	1	4 3 3 0	4 3 3 0	4 3 3 0
	3	4 4 3 2	4 4 3 2	4 4 3 2
	5	4 4 1 0	4 4 1 0	4 4 1 0
	7	4 3 2 0	4 3 2 0	4 3 2 0
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
3MM	0	0 0 0 0	0 0 0 0	0 0 0 0
Control	1	3 1 0 0	3 1 0 0	3 0 0 0
	3	3 3 2 0	3 3 2 0	3 3 2 0
	5	3 3 0 0	3 3 0 0	3 3 0 0
	7	2 0 0 0	2 0 0 0	2 0 0 0
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
5MM	0	0 0 0 0	0 0 0 0	0 0 0 0
Control	1	4 2 1 0	4 2 1 0	4 2 0 0
	3	3 2 0 0	3 2 0 0	3 2 0 0
	5	3 1 0 0	3 1 0 0	3 1 0 0
	7	4 3 0 0	4 3 0 0	4 3 0 0
	-14	0 0 0 0	0 0 0 0	0 0 0 0
	- 7	0 0 0 0	0 0 0 0	0 0 0 0
5X	0	0 0 0 0	0 0 0 0	0 0 0 0
V.P. Control	1	0 0 0 0	0 0 0 0	0 0 0 0
	3	0 0 0 0	0 0 0 0	0 0 0 0
	5	0 0 0 0	0 0 0 0	0 0 0 0
	7	0 0 0 0	0 0 0 0	0 0 0 0

EVALUATION STUDIES ON A CANINE ACUTE PHASE SERUM FRACTION ANALOGOUS TO HUMAN C-REACTIVE PROTEIN

by

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The detection of C-reactive protein (CRP) in the serums of humans during the acute phase of various inflammatory processes has been used as an aid to prognosis in the practice of human medicine for many years. A serum fraction analogous to human CRP, that cross-reacted with antiserum specific for the human fraction, was recently reported in dogs. This project was undertaken to evaluate the presence of this serum fraction in the dog and its relationship to inflammation. An agglutination reaction, using a latex-adsorbed rabbit antiserum specific for human CRP, was used to detect the presence of the cross-reacting canine serum fraction.

Thirty-one dogs, divided into 6 experimental groups, were used in this project. Each group was subjected to different inflammatory stimuli, and rectal temperatures and blood samples were taken prior to and following inoculation. A total of 283 temperatures and blood samples were obtained from the 6 groups. All dogs except those in Groups IV and V were euthanatized at the end of each experimental period and a gross and microscopic examination of the tissues was completed, with special emphasis on grossly inflamed tissues.

Each blood sample was divided into 2 portions: a portion that was allowed to clot from which serum was collected and an unclotted sample for hematologic analysis. Total and differential leukocyte count, hemoglobin level, and the packed cell volume were determined on the unclotted sample. The serum from the clotted sample was tested for the presence of CRP, exposed to temperatures from -20 C. to 70 C., and again tested for CRP in order to study the stability of the canine fraction for comparisons with its human counterpart. Each serum sample was subjected to

electrophoretic analysis so that any relationship between the inflammatory fraction and known serum fractions could be observed.

The data were tabulated and recorded graphically for comparison and correlation. Actual mean values were calculated for the CRP titers, rectal temperatures, total leukocyte counts, and serum fractions, to emphasize correlative trends and minimize individual variations.

Sixty-six of the 283 serums were positive when tested for CRP. Four of these reactions occurred at unexpected times during the experimental periods. A great deal of individual variation in CRP response to the inflammatory stimuli was observed. Considerable correlation of the mean CRP titers with the mean temperatures, total leukocyte counts, and beta globulin values was observed.

The canine acute phase serum fraction was stable when refrigerated overnight at 4 C. or stored for several days at -20 C. This serum fraction was inactivated by exposure to 70 C. for 30 minutes.

It was concluded that a canine serum fraction, analogous to human CRP, was present during the inflammatory processes. The results suggested that an antiserum specific for the canine inflammatory serum fraction could be an aid to prognosis in the practice of veterinary medicine and that further work should be done to develop and evaluate such a reagent.